DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Memorandum

Date NOV 26 1996

From Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of Roche Molecular Systems' AMPLICOR® Mycobacterium Tuberculosis Test

To The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

Kimber C. Richter

Kimber Richter, M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved ✓ Disapproved _____ Date _____

Prepared by: Roxanne Shively, CDRH, HFZ-440, 09/17/96, 594-2096

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

[DOCKET NO. _____]

Roche Molecular Systems, Inc.; PREMARKET APPROVAL OF AMPLICOR® Mycobacterium Tuberculosis Test

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Roche Molecular Systems, Inc., Somerville, N.J. for premarket approval, under the Federal Food, Drug, and Cosmetic Act (the act), of the AMPLICOR® Mycobacterium Tuberculosis Test. After reviewing the recommendation of the Microbiology Devices Panel, FDA's Center for Devices and Radiological Health (CDRH) notified the applicant, by letter on November 26, 1996, of the approval of the application.

DATES: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER); Written comments by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESSES: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review, and comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Sharon L. Hansen, Ph.D.,

Center for Devices and Radiological Health (HFZ-440),

Food and Drug Administration,

9200 Corporate Blvd.,

Rockville, MD 20850,

301-594-2096.

SUPPLEMENTARY INFORMATION: On December 22, 1994, Roche Molecular Systems, Inc., Somerville, N.J. 08876-3711, submitted to CDRH an application for premarket approval of the AMPLICOR® Mycobacterium Tuberculosis (MTB) Test. The device is a target amplified *in vitro* diagnostic test for the qualitative detection of *M. tuberculosis* complex DNA in concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens including bronchoalveolar lavages or aspirates, or tracheal aspirates. The AMPLICOR MTB Test is intended for use as an adjunctive test for evaluating acid fast bacilli (AFB) smear positive sediments prepared using NALC-NaOH or NaOH digestion-decontamination of respiratory specimens from untreated patients suspected of having tuberculosis. Untreated patients are patients who have: (1) received no antituberculosis therapy, (2) had less than 7 days of therapy, or (3) have not received such therapy in the last twelve months. Only untreated patients may be evaluated with this test. The AMPLICOR MTB Test should only be performed in institutions proficient in the culture and identification of *M. tuberculosis* (ATS Level II and III or CAP extent 3 and 4). The test should always be performed in conjunction with mycobacterial culture.

On January 25, 1996, the Microbiology Devices Panel of the Medical Devices Advisory Committee, an FDA advisory committee, reviewed and recommended approval of the application.

On November 26, 1996, CDRH approved the application by a letter to the applicant from the Director of the Office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

Opportunity for Administrative Review

Section 515(d)(3) of the act, (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act, for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices and procedures regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the

time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h), (21 U.S.C. 360e(d), 360j(h))) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated:_____.

_____

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

Mr. Alex Wesolowski
Roche Molecular Systems, Inc.
1080 U.S. Highway 202
Branchburg Township
Somerville, N.J. 08876-3711

NOV 26 1996

Re: P940040
AMPLICOR® Mycobacterium Tuberculosis Test
Filed: December 22, 1994
Amended: May 8, June 8, June 20, and December 14, 1995; January 16, March 1, July 1, August 6, August 23, October 9, October 24 and November 7, 1996

Dear Mr. Wesolowski:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the AMPLICOR® Mycobacterium Tuberculosis (MTB) Test. This device is a target amplified *in vitro* diagnostic test for the qualitative detection of *M. tuberculosis* complex DNA in concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens including bronchoalveolar lavages or aspirates, or tracheal aspirates. The AMPLICOR MTB Test is intended for use as an adjunctive test for evaluating acid fast bacilli (AFB) smear positive sediments prepared using NALC-NaOH or NaOH digestion-decontamination of respiratory specimens from untreated patients suspected of having tuberculosis. Untreated patients are patients who have: (1) received no antituberculosis therapy, (2) had less than 7 days of therapy, or (3) have not received such therapy in the last twelve months. Only untreated patients may be evaluated with this test. The AMPLICOR MTB Test should only be performed in institutions proficient in the culture and identification of *M. tuberculosis* (ATS Level II and III or CAP extent 3 and 4). The test should always be performed in conjunction with mycobacterial culture.

We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

FDA has also determined that to ensure the safe and effective use of the device that the device is further restricted within the meaning of section 520(e) under the authority of section 515(d)(1)(B)(ii), (1) insofar as the labeling specify the requirements that apply to the training of practitioners who may use the device as approved in this order; (2) insofar as the labeling specify the requirements that apply to laboratory facilities where the device is to be used as approved; and (3) insofar as the sale, distribution, and use must not violate sections 502(q) and (r) of the act.

In addition to the postapproval requirements in the enclosure, the postapproval reports must include the following information:

1. The results of customer monitoring and information/data requested by the FDA:

   a. AMPLICOR MTB test results, smear results, culture results, results of inhibition testing, and repeated replicate testing of specimens initially testing equivocal;

   b. Summaries of proficiency testing required of new users (laboratories) during the first 12 months of commercial distribution;

   c. A summary of the absorbance values obtained with the inhibition control.

   Available information/data should be submitted at 6 months and one year following the approval date and should include any data collected up to one month before the submittal date. After the first year following approval, the number and types of sites subject to continued and/or initial monitoring will be readdressed pending assessment of the data and information submitted for the initial 6 month monitoring period of the new user sites. These periodic reports should be submitted separately from the annual report.

2. All educational, promotional, and advertising materials in a periodic report within the first 6 months following approval and in the annual report thereafter;

3. A study protocol for evaluating the effects of bovine serum albumin used as a resuspension fluid, and other processing variables (NaOH and other resuspension fluids). The study design should be submitted as a periodic report within 3 months of approval and results submitted as a periodic report within 6 months of approval;

4. Revised end-product testing and stability testing to include an *M. tuberculosis* and MOTT organism sample, submitted as a revised manufacturing section supplement within 3 months of approval.

Expiration dating for this device has been established and approved at twelve months, based on the most labile component, Mycobacteria Master Mix. This is to advise you that the protocol you used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as provided by 21 CFR 814.39(a)(8).]

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Sharon L. Hansen, Ph.D. at (301) 594-2096.

Sincerely yours,

Kimber C. Richter

Kimber Richter, M.D.
Deputy Director, Clinical and
Review Policy
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

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CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the **addition** of, but **not the replacement** of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. **This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.**

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant**(s)** involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

(1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).

(2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:

(a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

(b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

(1) A mixup of the device or its labeling with another article.

(2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and

(a) has not been addressed by the device's labeling or

(b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.

(3) Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

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REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984, and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to FDA whenever they receive or otherwise became aware of information that reasonably suggests that one of its marketed devices

(1) may have caused or contributed to a death or serious injury or

(2) has malfunctioned and that the device or any other device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for this PMA, you shall submit the appropriate reports required by the MDR Regulation and identified with the PMA reference number to the following office:

Division of Surveillance Systems (HFZ-531)
Center for Devices and Radiological Health
Food and Drug Administration
1350 Piccard Drive, 340
Rockville, Maryland 20850
Telephone (301) 594-2735

Events included in periodic reports to the PMA that have also been reported under the MDR Regulation must be so identified in the periodic report to the PMA to prevent duplicative entry into FDA information systems.

Copies of the MDR Regulation and an FDA publication entitled, "An Overview of the Medical Device Reporting Regulation," are available by written request to the address below or by telephoning 1-800-638-2041.

Division of Small Manufacturers Assistance (HFZ-220)
Center for Devices and Radiological Health
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. General Information

Device Generic Name:	*In vitro* Nucleic Acid Amplificaton Test for the Direct Detection of *Mycobacterium tuberculosis*
Device Trade Name:	AMPLICOR® Mycobacterium Tuberculosis Test
Applicant's Name and Address:	Roche Molecular Systems 1080 US Highway 202 Branchburg Township Somerville, New Jersey 08876-3771

Premarket Approval Application (PMA) Number: P940040

Date of Panel Recommendation: January 25, 1996

Date of Notice of Approval to the Applicant: November 26, 1996

II. INDICATIONS FOR USE

The AMPLICOR® Mycobacterium Tuberculosis (MTB) Test is a target amplified *in vitro* diagnostic test for the qualitative detection of *M. tuberculosis* complex DNA in concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens including bronchoalveolar lavages or aspirates, or tracheal aspirates.

The AMPLICOR MTB Test is intended for use as an adjunctive test for evaluating acid fast bacilli (AFB) smear positive sediments prepared using NALC-NaOH or NaOH digestion-decontamination of respiratory specimens from untreated patients suspected of having tuberculosis. Untreated patients are patients who have: (1) received no antituberculosis therapy, (2) had less than 7 days of therapy, or (3) have not received such therapy in the last twelve months. Only untreated patients may be evaluated with this test. The AMPLICOR MTB Test should only be performed in institutions proficient in the culture and identification of *M. tuberculosis* (ATS Level II and III or CAP extent 3 and 4). The test should always be performed in conjunction with mycobacterial culture.

CONTRAINDICATIONS

There are no known contraindications for the AMPLICOR MTB Test.

WARNINGS AND PRECAUTIONS

Warnings and Precautions for use of the device are stated in the attached product labeling. (Attachment A)

1d

Background

Mycobacterium tuberculosis complex

M. tuberculosis complex includes the species *M. bovis*, *M. microti*, and *M. africanum*. However, *M. microti* infects only animals, *M. bovis* is uncommonly transmitted from infected animals to humans, and *M. africanum* causes pulmonary disease in humans in tropical Africa.[1,2] *M. tuberculosis* is by far the most common member of the complex that is responsible for human disease worldwide. Nontuberculous mycobacteria (mycobacteria other than *M. tuberculosis* complex, or MOTT) include *M. avium* complex (MAC) and other organisms that may also cause disease in humans.

M. tuberculosis complex and MOTT are presumptively identified in slides made from clinical specimens by their acid fast nature. All mycobacteria have cell walls with a high lipid content that prevents easy decolorization once stained (acid fastness). Growth rates are slow to very slow, with some species requiring supplementation for laboratory culture.[2] Definitive identification of *M. tuberculosis* and MOTT requires identification by traditional methods (observation of growth rate, colonial morphology, pigmentation and biochemical profiles), chromatographic analysis of lipid composition (thin layer chromatography, capillary gas chromatography, or high pressure liquid chromatography), or hybridization assays with specific nucleic acid probes.

M. tuberculosis complex organisms are a proven hazard to laboratory personnel as well as others who may be exposed to infectious aerosols in the laboratory. The infective dose for humans is low (ID_{50} <10 bacilli). Biosafety Level 2 practices are required for activities at the American Thoracic Society (ATS) laboratory Level I (preparation of AFB smears, collection and transport of mycobacterial specimens for culture). Biosafety Level 3 practices are required for laboratory activities of ATS levels II (isolation and identification of *M. tuberculosis*) and III (additionally performing susceptibility testing of *M. tuberculosis* isolates and identification of MOTT).[3, 9]

Tuberculosis

Tuberculosis is a bacterial disease caused by organisms of the *M. tuberculosis* complex; it is transmitted primarily by airborne droplet nuclei from individuals with pulmonary or laryngeal tuberculosis.[4] Tuberculosis (TB) can occur in any organ of the body, but only 5 to 15 percent of infected individuals will develop active disease within 2 years of primary infection. Pulmonary TB is still the primary manifestation in infected individuals who develop disease, but the incidence of extrapulmonary TB has progressively increased.[5] HIV-infected individuals who develop active TB have a high rate of extrapulmonary disease. HIV-positive patients with low CD4 counts tend to have radiographic presentations markedly different from the classical upper lobe cavitary disease of immunocompetent patients. Clinical disease due to reactivation of dormant TB is influenced by predisposing factors such as AIDS, malignancy, silicosis, immunosuppressive therapy, malnourishment, and other risk factors.[5]

Population groups in the United States that are at increased risk for infection with *M. tuberculosis* include medically underserved, low-income populations, immigrants from countries with a high

prevalence of TB, and residents of long-term-care and correctional facilities. Those at increased risk of developing disease following infection include individuals with HIV infection; close contacts of infectious cases; children less than 5 years old; patients with renal failure, silicosis, and diabetes mellitus; and individuals receiving treatment with immunosuppressive medications.[4]

The initial treatment of TB includes multiple antimicrobial agents, since administration of a single drug often leads to the development of resistance. *M. tuberculosis* becomes drug resistant through random, spontaneous genetic mutation. Susceptibility testing of the first isolate from all patients should be done to provide the physician a basis for therapeutic management, to identify emerging drug resistance, and to help monitor control efforts in areas where resistance is established. If culture positive sputum continues after three months of therapy, susceptibility testing should be repeated. During the first week of therapy, few patients convert from culture positive to culture negative.[6] Thereafter, patients responding to therapy will have significant reductions in organism loads and become culture negative. The time course until a patient becomes noninfectious is influenced by initial organism load, the presence of a drug-resistant strain, and the severity of coughing. Organism load reduction can be monitored with AFB smears; culturing is used to monitor bacteriologic sputum conversion, to assess response to therapy, and to monitor the emergence of resistant strains.[7]

After uniform national reporting of TB began in 1953, the number of cases reported annually declined steadily until 1985. Since that time TB has reemerged as a serious public health problem.[4] In addition, the development of multi-drug resistant strains of *M. tuberculosis* has become a major concern. Factors contributing to the increase in TB morbidity in the United States include an increase in foreign-born cases, the HIV/AIDS epidemic, and increased active TB transmission in higher risk populations.[8]

III. DEVICE DESCRIPTION

The AMPLICOR MTB Test incorporates the technologies of polymerase chain reaction (PCR), nucleic acid hybridization, and immunochemical photometric detection into an ELISA microwell plate format.

After liquefaction, decontamination, and concentration of respiratory specimens using NALC/NaOH or NaOH, 100 μL aliquots of the sediments are washed and heat-lysed to release cellular nucleic acids. The lysed sample is added to Master Mix reagent which contains the thermal stable *Taq* polymerase, the biotinylated primers, excess deoxynucleoside triphosphates, and added AmpErase® (uracil-N-glycosylase, UNG). During thermal cycling, DNA alternately denatures (during a heat cycle) and anneals with the biotinylated primers. The target DNA bracketed by the primers is a 584 base pair sequence of chromosomal DNA within the gene encoding the 16S ribosomal RNA that is conserved in mycobacteria and some related species.[24] Taq polymerase extends the annealed primers along the target templates to produce a DNA sequence termed an amplicon. This process is repeated for 35 cycles, each cycle effectively doubling the amount of amplicon DNA.

AmpErase®, a reagent added to the Master Mix reagent, recognizes and catalyzes the destruction of DNA containing deoxyuridine triphosphate that is always present in amplicons since deoxyuridine triphosphate is substituted for thymidine triphosphate in the Master mix reagent. AmpErase® is only active below 55°C. and acts during the first thermal cycling step to

inactivate up to 10^3 copies/reaction of deoxyuridine-containing amplicons. The remaining thermal cycling steps are maintained above 55°C. preventing destruction of newly generated amplicons. Prior to cooling, a denaturation solution inactivates residual AmpErase®. The use of AmpErase® minimizes contamination of samples with aerosolized amplicon from prior testing. Nevertheless, the AMPLICOR MTB Test must be performed with appropriate precautions and strict adherence to specified procedures to avoid other sources of contamination.

Following PCR amplification, amplicons are chemically denatured to form single stranded DNA. Aliquots of this mixture are then added to a microwell plate (MWP) containing a 21-base oligonucleotide probe specific for *M. tuberculosis* complex organisms. The biotin-labeled amplicon is captured by the probe coating the MWP, washed to remove unbound material, and Avidin-Horseradish Peroxidase Conjugate (Av-HRP) added. Av-HRP binds to the biotin-labeled amplicon captured by the target specific DNA probe. After additional washing, MWP-bound HRP catalyzes the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide to form a colored complex. The intensity (optical density) of the colored complex is measured by an automated microwell plate reader at a wavelength of 450 nm. Positive, negative, and equivocal results are determined by the level of the absorbance measurement.

The AMPLICOR MTB Test contains two controls: the *M. tuberculosis* Positive Control containing 20 copies/mL of non-infectious MTB DNA transcript which is used to simulate the MTB DNA target in the clinical specimen; and the *M. tuberculosis* Negative Control containing nonspecific DNA. A specimen lysis control prepared by testing laboratories is used to control the assay's specimen preparation procedures.

IV. ALTERNATIVE PRACTICES AND PROCEDURES

Tuberculin skin testing, radiography, assessment of physical findings, and identification of risk factors are used to determine patients with a high index of suspicion that TB may be present. AFB smears and cultures of clinical material are necessary to establish a definitive diagnosis of TB although a strong presumptive diagnosis may be made on radiographic findings when the patterns are typical.[9]

Definitive diagnosis of mycobacterial disease (except leprosy), including TB, requires growth of the microorganism. Although patients will be initially treated with a predetermined therapeutic regimen, cultures are also required for susceptibility testing to confirm the anticipated effectiveness of treatment. Culture for AFB is usually performed by inoculating several media with decontaminated sediment and incubating for up to 8 weeks. Conventional culture methodologies can detect *M. tuberculosis* growth as early as 1 week, but may take up to 8 weeks. Radiometric liquid culture, requires an average of 13 days to final culture result.[10] Current recommendations from CDC are to inoculate both a liquid medium and a solid medium.[4] After recovery of mycobacteria from culture media, identification of *M. tuberculosis* may be done by conventional biochemical testing, analysis of lipid content, or hybridization with specific DNA probes.

The American Thoracic Society (ATS), in collaboration with CDC, provides a classification scheme for TB that is based on pathogenesis and current treatment recommendations.[9] Patients with clinical suspicion of TB or positive AFB smears are reported to local health departments for

appropriate public health management (including contact investigations).[8] Final species identification and susceptibility results from positive cultures are also reported to the health department.

V. MARKETING HISTORY

The AMPLICOR MTB Test has been marketed in Australia, Austria, Belgium, Brazil, Canada, Denmark, France, Germany, Italy, Japan, Netherlands, New Zealand, Spain, Sweden, Switzerland, and the United Kingdom.

The AMPLICOR MTB Test has not been withdrawn from marketing in any country for reasons related to safety or effectiveness or for any other reason.

VI. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Prompt diagnosis of TB is critical, both to initiate appropriate therapy and to institute measures to prevent further exposures and spread of the disease to uninfected individuals, both in the community and health care facilities. A false positive result could lead to misdiagnosing a patient's medical status, resulting in the administration of unnecessary therapy and/or placing a patient in unwarranted isolation; additionally, the patient would be reported to the local health department for public health management, and contact investigations initiated. A false negative result could delay the correct diagnosis of TB and initiation of appropriate therapy. Delayed diagnosis can result in rapidly progressive disease, especially in HIV-positive patients and patients infected with multi-drug resistant strains of *M. tuberculosis*. In addition, the potentially infectious patient might not be placed into isolation until the results of culture were available.

False negative results could be caused by low organism load in the specimen, interfering substances, (See Table 3), presence of high numbers of MOTT, specimen processing errors, procedural errors, or the presence of inhibitors to the amplification reaction in the specimen. False positive results may be caused by sample contamination, carry-over contamination, sample misidentification, or potentially by cross reactivity with other species.

VII. SUMMARY OF STUDIES

A. Non-Clinical Studies

1. Establishment of the Cutoff Value and Validation of Assay Cut-Off

To determine the cut-off value for the AMPLICOR MTB Test, 303 frozen sputum specimens (160 AFB smear and/or *M. tuberculosis* culture positive; 143 AFB smear and culture negative) from symptomatic TB patients were tested. Cumulative Distribution Analysis of the data demonstrated that cut-off values of 0.25, 0.30, 0.35 and 0.40 A at 450 nm (A_{450}) provided 100 per cent sensitivity and 100 per cent specificity with this sample group. The majority (158/160) of positive absorbance values were >2.0 A_{450} in both replicates tested. The remaining 2 samples tested equivocal in one of two replicates; one sample was >2.0 and one <0.20 A_{450} in the other replicate.

To maximize the clinical sensitivity and specificity of the AMPLICOR MTB Test and allow for technical variations and precision of testing, an equivocal range (0.2 to 0.6 A_{450}) was established. When initial test results fall within this absorbance range, repeat duplicate testing is performed. During the clinical studies, the distribution of absorbance values for smear positive samples supported the cutoff and equivocal range derived from the preclinical testing. The majority of positive measurements were greater than 0.6 (97.8 percent or 131/134) and the majority of negative measurements were less than 0.2 (98.2 percent or 54/55) for the smear positive specimens tested. The distribution of these measurements is shown in Table 1.

TABLE 1: *AMPLICOR Test Results vs. Culture for Untreated Patients*
Concordant and Discrepant Specimen Results Selected By Histogram Interval and Categorized by AFB Smear Results

Absorbance Range (450 nm)	*Concordant Specimens*				*Discordant Specimens*				*Total No. Specimens*
	Smear (+)	*Smear (-)*	*Not Done*	*n*	*Smear (+)*	*Smear (-)*	*Not Done*	*n*	
0.000 - 0.100	29	3729	4	3762	6	42	0	48	3810
0.100 - 0.200	18	121	0	139	1	0	0	1	140
0.200 - 0.300	1	5	0	6	0	0	0	0	6
0.300 - 0.400	0	3	0	3	0	2	0	2	5
0.400 - 0.500	3	0	0	3	0	0	0	0	3
0.500 - 0.600	0	0	0	0	0	1	0	1	1
0.600 - 0.800	0	1	0	1	0	2	0	2	3
0.800 - 1.000	0	0	0	0	0	3	0	3	3
1.000 - 1.200	0	0	0	0	0	0	0	0	0
1.200 - 1.400	1	2	0	3	0	1	0	1	4
1.400 - 1.600	1	2	0	3	0	3	0	3	6
1.600 - 1.800	1	3	0	4	0	1	0	1	5
1.800 - 2.000	0	2	0	2	0	0	0	0	2
2.000 - 2.500	4	5	0	9	0	3	0	3	12
2.500 - 3.000	18	6	0	24	0	4	0	4	28
3.000 - 3.500	50	7	0	57	0	5	1	6	63
3.500 - 4.000	55	8	0	63	1	1	0	2	65
TOTAL	181	3894	4	4079	8	68	1	77	4156

2. Analytical Specificity

The analytical specificity of the AMPLICOR MTB Test was evaluated by testing bacterial and viral strains obtained primarily from the ATCC and from some clinical isolates. Of the 153 microbial strains tested, including 48 mycobacterial strains representing 34 species, only the 7 strains of the *M. tuberculosis* complex (1 x *M. bovis*, 2 x *M. microti*, 2 x *M tuberculosis*, 2 x *M. africanum*) had positive values in the AMPLICOR MTB Test. The remaining 146 strains, comprising 41 strains of non-tuberculosis mycobacteria, 96 other bacterial and fungal strains, and 9 viruses were consistently negative. The strains tested are shown in Table 2.

Table 2: Amplicor Mycobacterium Tuberculosis Specificity Testing

ATCC #	Organism	Rep. #1	Rep. #2	Rep. #1	Rep. #2
		(4x10 ^{7} cells/mL)		(4x10 ^{4} cells/mL)	
		ABS at 450 nm		ABS at 450 nm	
23055	Acinetobacter calcoaceticus	0.092	0.090	0.057	0.062
19425	Actinomadura madurae	0.070	0.075	0.067	0.059
19411	Actinomyces pyogenes	0.124	0.102	0.052	0.050
27366	Actinoplanes italicus	0.108	0.059	0.053	0.052
7966	Aeromonas hydrophila	0.060	0.058	0.061	0.072
9345	Arcanobacterium haemolyticum	0.075	0.066	0.054	0.052
14358	Arthrobacter oxydans	0.082	0.107	0.061	0.052
6051	Bacillus subtilis	0.180	0.107	0.057	0.059
25285	Bacteriodes fragilis	0.071	0.074	0.073	0.081
60915	Blastomyces dermatitidis	0.086	0.120	0.087	0.055
15311	Bordetella parapertussis	0.058	0.095	0.053	0.060
9797	Bordetella pertussis	0.090	0.070	0.052	0.051
25238	Branhamella (Moraxella) catarrhalis	0.084	0.122	0.064	0.071
8377	Brevibacterium linens	0.093	0.061	0.059	0.058
33560	Campylobacter jejuni	0.081	0.087	0.053	0.058
18804	Candida albicans	0.056	0.060	0.106	0.076
VR348B	Chlamydia trachomatis	0.102	0.080	0.087	0.146
12472	Chromobacterium violaceum	0.078	0.062	0.058	0.063
8090	Citrobacter freundii	0.086	0.056	0.054	0.049
13124	Clostridium perfringens	0.053	0.047	0.057	0.053
—	Coccidioides immitis	0.081	0.064	0.070	0.064
14665	Corynebacterium aquaticum	0.103	0.081	0.075	0.067
11913	Corynebacterium diphtheriae	0.059	0.066	0.072	0.061
27010	Corynebacterium diphtheriae	0.054	0.057	0.061	0.062
19409	Corynebacterium diphtheriae	0.115	0.072	0.091	0.086
10340	Corynebacterium flavescens	0.098	0.052	0.082	0.055
13032	Corynebacterium glutamicum	0.071	0.060	0.053	0.053
43734	Corynebacterium jeikeium	0.072	0.182	0.123	0.125
23347	Corynebacterium minutissimum	0.075	0.085	0.059	0.070
10700	Corynebacterium pseudodiphtheriticum	0.127	0.074	0.066	0.058
10701	Corynebacterium pseudodiphtheriticum	0.089	0.086	0.062	0.069
19410	Corynebacterium pseudotuberculosis	0.073	0.072	0.067	0.059
19412	Corynebacterium renale	0.165	0.104	0.078	0.070
6940	Corynebacterium striatum	0.063	0.059	0.058	0.064
373	Corynebacterium xerosis	0.086	0.090	0.068	0.063
32045	Cryptococcus neoformans	0.072	0.095	0.068	0.054
13939	Deinococcus radiodurans	0.062	0.077	0.060	0.054
14637	Dermatophilus congolensis	0.069	0.104	0.073	0.075
15994	Derxia gummosa	0.091	0.100	0.079	0.063
23834	Eikenella corrodens	0.079	0.079	0.062	0.062
13048	Enterobacter aerogenes	0.189	0.075	0.057	0.058
13047	Enterobacter cloacae	0.055	0.072	0.057	0.061
19433	Enterococcus faecalis	0.055	0.061	0.068	0.084
19434	Enterococcus faecium	0.068	0.059	0.074	0.077
11775	Escherichia coli	0.098	0.074	0.059	0.061
25586	Fusobacterium nucleatum	0.094	0.092	0.060	0.072

33610	Gordona sputi	0.075	0.070	0.063	0.067
33391	Haemophilus influenza	0.064	0.051	0.053	0.050
33392	Haemophilus parainfluenzae	0.053	0.099	0.057	0.059
—	Histoplasma capsulatum	0.101	0.066	0.137	0.054
13883	Klebsiella pneumoniae	0.072	0.143	0.054	0.066
11296	Klebsiella pneumoniae subsp. ozaenae	0.067	0.059	0.056	0.058
11578	Lactobacillus casei	0.075	0.063	0.085	0.064
33204	Legionella micdadei	0.069	0.183	0.073	0.072
33152	Legionella pneumophila	0.080	0.059	0.053	0.060
8180	Microbacterium lactamica	0.147	0.084	0.060	0.064
25420	Mycobacterium africanum	4.000	4.000	4.000	4.000
35711	Mycobacterium africanum	4.000	4.000	4.000	4.000
25276	Mycobacterium asiaticum	0.084	0.130	0.091	0.105
23366	Mycobacterium aurum	0.120	0.118	0.110	0.127
25291	Mycobacterium avium	0.111	0.125	0.103	0.115
19210	Mycobacterium bovis	4.000	4.000	4.000	4.000
19627	Mycobacterium chitae	0.073	0.091	0.069	0.063
49103	Mycobacterium cookii	0.059	0.063	0.067	0.060
35219	Mycobacterium fallax	0.092	0.106	0.099	0.108
35219	Mycobacterium fallax	0.090	0.096	0.095	0.067
14474	Mycobacterium flavescens	0.079	0.091	0.055	0.070
6841	Mycobacterium fortuitum	0.073	0.101	0.092	0.103
15754	Mycobacterium gastri	0.076	0.155	0.103	0.096
14470	Mycobacterium gordonae	0.087	0.110	0.109	0.099
13950	Mycobacterium intracellulare	0.115	0.129	0.106	0.096
12478	Mycobacterium kansasii	0.100	0.128	0.105	0.111
33013	Mycobacterium komassense	0.058	0.066	0.079	0.078
29571	Mycobacterium malmoense	0.077	0.133	0.099	0.095
11565	Mycobacterium marinum	0.129	0.111	0.069	0.093
927	Mycobacterium marinum	0.120	0.144	0.157	0.207
29254	Mycobacterium marinum	0.144	0.123	0.154	0.158
15069	Mycobacterium marinum	0.100	0.114	0.137	0.139
11566	Mycobacterium marinum	0.099	0.113	0.119	0.106
25039	Mycobacterium marinum (CMCC 4090)	0.103	0.109	0.104	0.116
11152	Mycobacterium microti	4.000	4.000	4.000	4.000
19422	Mycobacterium microti	4.000	4.000	4.000	4.000
25795	Mycobacterium neoaurum	0.068	0.108	0.096	0.090
19530	Mycobacterium nonchromogenicum	0.090	0.065	0.075	0.077
11758	Mycobacterium phlei	0.072	0.113	0.095	0.092
19981	Mycobacterium scrofulaceum	0.087	0.128	0.115	0.099
19420	Mycobacterium smegmatis	0.069	0.105	0.098	0.096
33027	Mycobacterium sphagni	0.076	0.100	0.098	0.107
35799	Mycobacterium szulgai	0.094	0.153	0.106	0.110
15755	Mycobacterium terrae	0.069	0.108	0.090	0.121
19527	Mycobacterium thermoresistibile	0.068	0.096	0.100	0.085
23292	Mycobacterium triviale	0.130	0.094	0.063	0.063
27294	Mycobacterium tuberculosis	4.000	4.000	4.000	4.000
—	Mycobacterium tuberculosis(CMC3895)	4.000	4.000	4.000	4.000
19250	Mycobacterium xenopi	0.105	0.168	0.118	0.120
23114	Mycoplasma hominis	0.084	0.081	0.052	0.070
15531	Mycoplasma pneumoniae	0.064	0.065	0.050	0.136
19424	Neisseria gonorrhoeae	0.055	0.059	0.065	0.068
23970	Neisseria lactamica	0.070	0.055	0.102	0.055
13102	Neisseria meningitidis	0.170	0.089	0.054	0.056

19247	Nocardia asteroides	0.142	0.090	0.074	0.049
19296	Nocardia brasiliensis	0.055	0.092	0.057	0.063
—	Nocardia farcinica (W5218)	0.065	0.070	0.096	0.079
—	Nocardia nova (W5194)	0.062	0.071	0.075	0.154
14629	Nocardia otitidiscaviarum	0.085	0.122	0.070	0.088
—	Nocardia transvalensi (W4692)	0.062	0.106	0.065	0.075
25835	Oerskovia turbata	0.097	0.084	0.057	0.077
27731	Peptococcus niger	0.090	0.087	0.062	0.070
27337	Peptostreptococcus anaerobius	0.075	0.071	0.059	0.081
15794	Peptostreptococcus magnus	0.120	0.132	0.066	0.065
—	Pneumocycstis carinii 28914 (10^6 copies)	0.087	0.072	0.087	0.067
25260	Porphyromonas asaccharolytica	0.092	0.099	0.058	0.068
33277	Porphyromonas gingivalis	0.080	0.109	0.061	0.076
25845	Prevotella melaninogenica (CMCC 2833)	0.079	0.117	0.059	0.065
6919	Propionibacterium acnes	0.074	0.077	0.054	0.056
29906	Proteus mirabilis	0.090	0.155	0.063	0.065
10145	Pseudomonas aeruginosa	0.078	0.094	0.060	0.053
33611	Rhodococcus aichiensis	0.125	0.086	0.068	0.064
25592	Rhodococcus bronchialis	0.088	0.092	0.077	0.073
33609	Rhodococcus chubuensis	0.093	0.099	0.067	0.069
6939	Rhodococcus equi	0.061	0.066	0.065	0.077
19430	Salmonella choleraesuis subsp. choleraesuis	0.110	0.078	0.078	0.055
13076	Salmonella choleraesuis subsp. choleraesuis	0.052	0.083	0.053	0.062
—	Serratia marcescens	0.086	0.081	0.065	0.070
12598	Staphylococcus aureus	0.068	0.085	0.062	0.073
14990	Staphylococcus epidermidis	0.061	0.066	0.068	0.070
23915	Streptomyces griseinus	0.114	0.076	0.116	0.133
17744	*Veillonella atypica*	0.056	0.080	0.064	0.076
10790	Veillonella parvula	0.077	0.055	0.071	0.105
17802	Vibrio parahaemolyticus	0.132	0.087	0.056	0.094
13637	Xanthomonas maltophilia	0.089	0.059	0.087	0.094
9610	Yersinia enterocolitica	0.120	0.088	0.059	0.065

Source	Organism	Type of Sample	Copies/PCR (CFU or PFU/PCR)	OD at 450 nm Rep #1	Rep #2
RMSCC 2975	M. celatum	Bacteria	1.00E+06	0.057	0.058
-	*M. leprae*	DNA	1.00E+06	0.061	0.059
90A-380	*M. terrae*	DNA	1.00E+06	0.073	0.072
90A-7889	*M. terrae*	DNA	1.00E+06	0.051	0.060
87A-0487	*M. terrae*	DNA	1.00E+06	0.067	0.073
91A-1818	*M. genavense*	DNA	1.00E+06	0.046	0.043
89A-6336	*M. genavense*	DNA	1.00E+06	0.039	0.041
89A-2120	*M. genavense*	DNA	1.00E+06	0.041	0.042
89A-6337	*M. genavense*	DNA	1.00E+06	0.042	0.045
VR-955	Respiratory Syncytial	Virus	2.80E+01 PFU	0.041	0.039
VR-1310	*C. pneumonia*	Bacteria	1.80E+02 PFU	0.041	0.040
ATCC 12344	*S. pyogenes*	Bacteria	1.00E+06	0.039	0.039
ATCC 33400	*S. pneumoniae*	Bacteria	1.00E+06	0.040	0.041

Viral Isolates

ATCC #	Classification	#PFUs/50μ	Rep. #1	Rep. #2
VR-7	Adenovirus	$3.2x10^5$	0.064	0.057
VR-977	*Cytomegalovirus*	$9.8x10^1$	0.059	0.076
VR-1044	Enterovirus	$5.6x10^5$	0.049	0.063
VR-539	*Herpes Simplex I*	$5.6x10^5$	0.093	0.133
VR-523	*Influenza B*	Unknown	0.059	0.066
VR-92	*Parainfluenza 2*	$2.8x10^5$	0.063	0.078
VR-955	*Respiratory Syncytial Virus*	$2.8x10^1$	0.065	0.074
VR-284	*Rhinovirus 14*	$2.8x10^4$	0.130	0.081

3. Analytical Sensitivity (Limit of Detection)

Using purified *M. tuberculosis* DNA, the AMPLICOR MTB Test was able to reproducibly detect 5 or more copies (as determined by Poisson analysis) of target per reaction.

Thirty-eight *M. tuberculosis* strains obtained from geographically diverse clinical isolates were serially diluted in negative sputum, colony counts of dilutions determined, and the AMPLICOR MTB Test was performed. Thirty-five strains were reproducibly detected (absorbance greater than 0.35) with those dilutions of *M. tuberculosis* at a level of $\geq$ 450 CFU/ mL ($\geq$ 10 CFU/PCR reaction). Three multiply-drug resistant strains failed to grow in colony count assessments. One was reproducibly detected in 2 dilutions; the other two isolates were not reproducibly detected in any dilution, however amplification and detection was documented in 4/18 and 2/18 testings. These strains could not be retrieved for retesting and it is unknown whether the inconsistent detection was due to dilutions containing few or no intact organisms.

Since non-viable organisms could not be enumerated in this study and probably contributed to lower CFU detection limits, and several strains showed variability in the 450-800 CFU range, a limit of detection of 800 CFU/mL (20 CFU/ reaction) is claimed for the AMPLICOR MTB Test. In the analysis of the AMPLICOR MTB Test results in the clinical study data, sensitivity was reduced in smear negative specimens and the use of the assay was limited to those specimens that were determined to be AFB smear positive, with the probablility of having minimally 10^4 organisms/mL specimen.

4. Interference Studies

Endogenous and exogenous substances and closely related organisms that may be present in clinical respiratory specimens were tested in the AMPLICOR MTB Test with and without added DNA target (20 copies *M. tuberculosis* DNA/reaction). The bronchial dilators (Primatene®, Proventil® and Vanceril®) were found not to interfere with AMPLICOR MTB Test measurements at ten times normal dosages. Similarly, sputum induction solution (3 per cent NaCl) did not interfere with assay results.

White blood cells at 1 x 10^7 cells/mL did decrease the measured absorbances with the AMPLICOR MTB Test for 20 copies genomic DNA (see Table 3). Blood up to 50 percent (volume/volume) had no interfering effect with 20 copies genomic DNA.

TABLE 3: Interference of White Blood Cells in the AMPLICOR Mycobacterium Tuberculosis Test

Replicate	No Added WBC	1.00E+06 WBC/mL	1.00E+07 WBC/mL	1.00E+08 WBC/mL
No DNA Spiked into Working Master Mix				
1	0.044	0.041	0.042	0.044
2	0.043	0.039	0.047	0.053
3	0.042	0.049	0.042	0.044
Mean	0.043	0.043	0.044	0.047
Std Dev	0.001	0.005	0.003	0.005
% CV	2.3	12.3	6.6	11.0
***M. tuberculosis* Genomic DNA Spiked into Master Mix (20 copies/PCR)**				
1	4.000	4.000	2.729	0.041
2	4.000	4.000	1.690	0.046
3	4.000	4.000	1.653	0.046
Mean	4.000	4.000	2.024	0.044
Std Dev	N/A	N/A	0.611	0.003
% CV	N/A	N/A	30.2	6.5
AMPLICOR MTB Positive Control Spiked into Master Mix (20 copies/PCR)				
1	4.000	4.000	4.000	0.041
2	4.000	4.000	4.000	0.039
3	4.000	4.000	4.000	0.046
Mean	4.000	4.000	4.000	0.042
Std Dev	N/A	N/A	N/A	0.004
% CV	N/A	N/A	N/A	8.6

The AMPLICOR MTB Test is specific for the detection of the *M. tuberculosis* complex by virtue of the oligonucleotide probe coated on the detection plates. However, because the nucleic acid primers used in the amplification reaction are genus-specific, large numbers of MOTT or other closely related organisms may cause false negative results due to competitive amplification. *M. avium*, *M. intercellulare*, *M. kansasii*, and *M. gordonae* as well as various *Corynebacterium spp.*, *Gordona sputi*, and *Rhodococcus bronchialis* reduced the AMPLICOR MTB Test absorbances for 20 copies of *M. tuberculosis* genomic DNA (See Table 4).

TABLE 4: AMPLICOR Mycobacterium Tuberculosis Test Bacterial Interference Studies

Organism	ATCC Number	Level Causing False Negative MTB Result at 20 copies MTB/PCR
Corynebacterium pseudodiphtheriticum	10700	1.00E+06 CFU/mL
Corynebacterium pseudodiphtheriticum	10701	1.00E+07 CFU/mL
Corynebacterium pseudotuberculosis	19410	1.00E+08 CFU/mL
Corynebacterium renale	19412	> 1.00E+08 CFU/mL
Gordona sputi	33610	1.00E+08 CFU/mL
Mycobacterium avium	25291	1.00E+05 CFU/mL
Mycobacterium gordonae	14470	1.00E+08 CFU/mL
Mycobacterium intracellulare	13950	1.00E+07 CFU/mL
Mycobacterium kansasii	12478	1.00E+07 CFU/mL
Neisseria lactamica	23970	> 1.00E+08 CFU/mL
Rhodococcus bronchialis	25592	1.00E+07 CFU/mL

Analysis of AMPLICOR MTB Test results for different specimen characteristics observed and documented for specimens tested during the clinical studies supported the absence of significant interference in specific specimen types. A summary of this data is shown in Table 5. The specific occurrence of inhibition that would result in a false negative result was assessed only in specimens that were considered discrepant during the clinical studies. Since this analysis included specimens from treated and untreated patients and from smear positive and smear negative specimens, conclusions could not be drawn. Of the 7 specimens that were from untreated patients and were smear positive and culture positive with a negative AMPLICOR MTB Test, five were tested retrospectively from frozen samples by spike-in testing and repeat testing with AMPLICOR MTB Test. Four remained negative in the repeat test, and two were inhibitory by spike-in testing.

TABLE 5: Significance of Interferents with False Negative Results

Observation/ Potential Interferent	Present Yes/No	TB Culture Positive Specimens Replicate #1 False Negatives			Replicate #2 False Negatives		
		n/N	%	p Value	n/N	%	p Value
Mucus	No	73/411	17.8		79/411	19.2	
	Yes	13/43	30.2	0.063	12/43	27.9	0.227
Blood	No	82/446	18.4		88/446	19.7	
	Yes	4/8	50.0	0.046	3/8	37.5	0.203
Purulence	No	83/401	20.7		86/401	21.5	
	Yes	3/53	5.7	0.008	5/53	9.4	0.044
Particulates	No	81/426	19.0		86/426	20.2	
	Yes	5/28	17.9	-1.00	5/28	17.9	-1.00
Watery Specimen	No	66/389	17.0		70/389	18.0	
	Yes	20/65	30.8	0.016	21/65	32.3	0.011

5. Validation of Decontamination Methods

A study was conducted to determine if the AmpErase® used in the AMPLICOR MTB Test was effective in selectively destroying amplicons at levels of 10^2 - 10^7 copies/PCR reaction. Several concentrations (10^2 - 10^7 copies/PCR reaction) of amplified *M. tuberculosis* DNA containing uracil in place of thymidine were tested in the AMPLICOR MTB Test amplification reaction. Additionally, to evaluate the possibility of add-back inhibition resulting from the presence of large amounts of amplicon in the amplification mixture, *M. tuberculosis* amplicons at levels of 10^2 - 10^7 copies/PCR reaction were spiked into an amplification reaction containing 20 copies/PCR of a non-*M. tuberculosis* DNA template.

In this study, AmpErase® eliminated false positive results caused by amplicon contamination at levels up to 10^3 amplicon copies/reaction. This represents a level of potential aerosol contamination of samples and/or reagents by amplicons generated in previous tests. Add-back inhibition was found to occur at amplicon levels above 10^4 copies/PCR reaction.

6. Validation of Transport and Storage Conditions

Effects of different specimen transport and storage conditions were evaluated using freshly collected specimens and simulated specimens prepared to contain known levels of *M. tuberculosis* organisms. In one study, 211 clinical specimens including 13 *M. tuberculosis* culture positive specimens, were tested the on the day of digestion-decontamination-concentration, after 4 or 7 days storage at 2-8°C, and after freezing at -70°C. For the 15 AMPLICOR MTB Test positive specimens, the comparison of mean absorbances on Day 0 and day 7 was significantly different (p=0.0292). No statistical difference in mean absorbances was seen with the positive specimens tested on Day 4.

Additionally, 25 simulated positive samples were tested after storage at -70°C, 2-8°C, 25°C, and 45°C. Lysates for 22 of these samples were also tested stored under the same conditions. The effects of multiple freeze-thaw cycles was also evaluated with 12 of the simulated positive samples. The resulting data showed that decontaminated specimens are stable for up to 4 days when stored at 2-8°C, or up to 8 months when stored at -70°C. AMPLICOR lysates (liquefied, decontaminated and concentrated respiratory specimens which have been processed by the AMPLICOR Specimen Preparation Procedure) are stable at 2° to 8°C for up to 4 days or -70°C for up to 6 months. The package insert will recommend storage for repeat testing and retrieval for up to 6 months for either decontaminated specimens or lysates.

7. Non-Infectivity of Test Materials

The effects of the AMPLICOR MTB Test specimen processing procedure on viability of mycobacterial organisms was evaluated using a total of 340 sputum specimens, including 300 specimens collected prospectively and 40 retrospective specimens that had been stored frozen. Of the 340 specimens in the study, 223 were found to be culture negative for *M. tuberculosis* prior to AMPLICOR MTB Test specimen processing, 16 were reported as contaminated, and 101 were mycobacterial culture positive. Following the AMPLICOR

MTB Test specimen processing procedure, none of the 340 specimens were culture positive for *M. tuberculosis* or any other mycobacterial species. Nine of the culture positive specimens were documented to contain numerous AFB by microscopy.

8. Precision

The Within-run, Between-run, Between-Day, Between-Site and Total Precision of the AMPLICOR MTB Test were evaluated by following the procedures contained in the NCCLS EP5T2 Guideline.[11] The samples used for the study were simulated patient samples prepared by spiking decontaminated sputum with *M. tuberculosis*. The *M. tuberculosis* levels in the simulated specimens were quantitated by culture and found to be approximately 0, 450, 950, and 1300 CFU/mL. Testing was performed twice per day on three days (consecutive where possible) at four sites. The results of these studies are summarized in Table 6.

Table 6: AMPLICOR MTB Test Precision Study

SAMPLE:	0 CFU/mL	450 CFU/mL	950 CFU/mL	1300 CFU/mL
No. of Replicates	282	288	288	288
% Correct	100%	100%	100%	100%
Mean A_{450}	0.06	3.10	3.47	3.63
Between Day SD	0.003	0.086	0.083	0.125
Between Day CV	4.83%	2.77%	2.40%	3.44%
Between Run SD	0.002	0.353	0.236	0.176
Between Run CV	3.83%	11.37%	6.80%	4.85%
Within Run SD	0.012	0.535	0.298	0.241
Within Run CV	19.97%	17.23%	8.60%	6.64%
Total SD	0.0126	0.646	0.390	0.324
Total CV	20.9%	20.8%	11.22%	8.9%

9. Reproducibility

The reproducibility of the AMPLICOR MTB Test was determined in a study at three clinical laboratories that had no prior experience in the use of the test procedure. A panel consisting of masked simulated clinical specimens was tested at each laboratory. The simulated specimens were prepared by spiking decontaminated sputum with an avirulent strain of *M. tuberculosis* (ATCC# 25177). The spiked specimens were quantitated by culture and found to contain approximately 0, 450, 950, and 1300 CFU/mL. The AMPLICOR MTB Test demonstrated 100 per cent reproducibility when concentrations $\geq$ 450 CFU/mL (equivalent to $\geq$ 11 CFU/PCR) were tested.

For negative samples, 99.3% (143/144) were correctly identified. The results of the testing performed at the 3 sites are presented in Table 7.

Table 7: AMPLICOR MTB Test Reproducibility

SITE	0 CFU/mL	450 CFU/mL	950 CFU/mL	1300 CFU/mL
Site 1	100% (36/36)	100% (6/6)	100% (6/6)	100% (6/6)
Site 2	100% (36/36)	100% (6/6)	100% (6/6)	100% (6/6)
Site 3*	97.2% (35/36)	100% (6/6)	100% (6/6)	100% (6/6)
Total	99.3 % (143/144)	100% (24/24)	100% (24/24)	100% (24/24)

* Upon repeat, Site 3 had no false positive test results for the 0 CFU/mL specimen

B. Clinical Studies

1. Clinical Study Objectives

Ten investigational sites representing diverse geographic regions and patient populations participated in a clinical study to evaluate the AMPLICOR MTB Test compared to mycobacterial culture methods for the identification of specimens with *M. tuberculosis*. The participating centers included seven large urban medical facilities, one public health center, one small community hospital, and one specialized respiratory disease center. Each site collected patient information and performed laboratory testing with the Amplicor.

Grady Memorial Hospital
Emory University
Atlanta, GA

San Francisco General Hospital
University of California, San Francisco
San Francisco, CA

Diagnostic Services, Inc.
Naples Community Hospital
Naples, FL.

University of British Columbia Provincial Laboratory
Vancouver, B.C. Canada

Southwestern Medical Center
University of Texas
Dallas, TX

Catholic Medical Center
Mary Immaculate Hospital
NY, NY

Orange County Public Health & Medical Services
Santa Ana, CA

University of Southern California Pathology Reference Laboratory - USC/LAC Medical Center
Los Angeles, CA

Thomas Jefferson University Hospital
Medicine Philadelphia, PA

Nat'l Jewish Ctr for Immunology and Respiratory
Denver, CO

A total of 2559 patients were entered into the clinical study for the AMPLICOR MTB Test. From these patients, 5805 respiratory specimens were collected and evaluated for AFB smear and culture positivity. The following patient groups were included in the clinical

study: HIV infected, close contacts of infectious TB cases, health care workers, high-risk medical conditions, foreign-born individuals (from high prevalence countries), BCG vaccinated, alcoholics, intravenous drug users, homeless individuals, residents of long-term care facilities, prisoners, immunocompromised patients, diagnosed with AIDS, organ transplantation, oncology, and clinically diagnosed multiple drug resistant TB cases. Specimens were collected from September, 1993 through November, 1993.

Of the total patients and specimens, 4156 specimens were collected from 1833 untreated patients. Untreated patients were defined as patients who were antimycobacterial treatment naive, on antimycobacterial treatment for less than 7 days, or had not received antimycobacterial treatment for the previous 12 months. Only specimens collected from untreated patients that were AFB smear positive were used in the data analyses. The prevalence of TB in the untreated patient population averaged 5.3 per cent and ranged from 1.7 per cent to 12 per cent across the ten clinical sites.

Within the untreated patient group, 189 AFB smear positive specimens were collected from 95 patients. Of these specimens, 134 were positive for *M. tuberculosis* complex by the AMPLICOR MTB Test and by mycobacterial culture. Four of the MTB culture positive specimens were also MOTT culture positive. Fifty five of the smear positive specimens were negative for *M. tuberculosis* complex by the AMPLICOR MTB Test. Of the 55 smear positive/AMPLICOR negative specimens, 7 were culture positive for *M. tuberculosis* complex and 48 were culture negative for *M. tuberculosis* complex. Two of these 7 AMPLICOR MTB Test false negative samples were demonstrated to have inhibitors by spike-in testing (the addition of target nucleic acid (DNA) into a duplicate test for a specimen to act as an internal control for determining the presence of inhibitors in the specimen that could prohibit amplification or detection of target in the specimen) from archived frozen sample. Thirty-nine of the 48 smear positive/MTB culture negative/AMPLICOR MTB Test negative specimens were MOTT culture positive and included the following isolates: MAI complex, *M. kansasii*, *M. xenopi*, *M. fortuitum*, *M. gordonae*, and unidentified rapid growers. The remaining nine specimens were culture negative, failing to grow either *M. tuberculosis* or MOTT.

Performance of the AMPLICOR MTB Test in smear negative specimens was also evaluated, but confounding variables of low organism load, inhibition, and prevalence effects prevented a determination of safety and effectiveness.

Table 8 shows the clinical performance of the AMPLICOR MTB Test by clinical study site for AFB smear positive specimens obtained from untreated patients as compared to composite culture results for these patients (any culture *M. tuberculosis* complex positive for that patient before treatment or within the first week of treatment was considered indication of a positive patient status for TB). Table 9 shows the overall clinical performance of the AMPLICOR MTB Test versus composite culture results.

Table 8: Smear Positive Specimens Collected from Untreated Patients Compared to Composite Culture Results

Parameter	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Total
TB Prevalence (%)	2.2	4.3	7.1	1.7	3.3	11.3	4.6	12.0	2.0	5.2	5.3
Sensitivity (%)	100 (9/9)	100 (4/4)	98.2 (54/55)	0 (0/1)	100 (4/4)	93.3 (14/15)	100 (6/6)	90.9 (20/22)	100 (5/5)	88.9 (16/18)	95.0 (134/141)
PPV (%)	100 (10/10)	100 (5/5)	100 (54/54)	NA (0/0)	100 (4/4)	100 (14/14)	100 (6/6)	100 (20/20)	100 (5/5)	100 (16/16)	100 (134/134)
Specificity (%)	100 (13/13)	100 (1/1)	100 (10/10)	100 (7/7)	100 (1/1)	100 (6/6)	100 (1/1)	100 (7/7)	100 (2/2)	NA (0/0)	100 (48/48)
NPV (%)	100 (13/13)	100 (1/1)	90.9 (10/11)	87.5 (7/8)	100 (1/1)	85.7 (6/7)	100 (1/1)	77.8 (7/9)	100 (2/2)	0 (0/2)	87.3 (48/55)

Table 9: Smear Positive Specimens Collected from Untreated Patients Compared to Composite Culture Results

		MTB Composite Culture Result	
		+	-
AMPLICOR Result	+	134	0
	-	7	48

VIII. CONCLUSIONS DRAWN FROM THE STUDIES

The non-clinical study data showed that the AMPLICOR MTB Test had no cross-reactivity with a range of microbial organisms tested. The AMPLICOR MTB Test also had analytical sensitivity which allowed for a limit of detection for the *M. tuberculosis* complex appropriate for AFB smear positive specimens. The AMPLICOR MTB Test showed no interference from endogenous and exogenous substances, with the exception of human WBCs. The level at which this interference was demonstrated would not be clinically significant except in rare specimen types. The studies done also demonstrated acceptable precision and reproducibility, and effectiveness of controls for monitoring assay parameters.

The assay cutoff, using an equivocal region, was validated by statistical methods and in the clinical studies. To further maximize the clinical accuracy of the test, the use of an equivocal range around the cutoff was established. The transport and storage conditions for clinical specimens, processed clinical specimens, and AMPLICOR lysates were established and validated in clinical and non-clinical studies.

The data from the clinical study showed that the AMPLICOR MTB Test had a high clinical sensitivity and specificity for the detection of the *M. tuberculosis* complex in AFB smear positive specimens when compared to standard culture procedures. The AMPLICOR MTB Test was shown to have a clinical sensitivity of 95 per cent (95 percent confidence interval: 91.4-98.6) and a clinical specificity of 100 per cent (95 percent confidence interval: 97.8-100) for the detection of the *M. tuberculosis* complex in specimens when compared to composite culture results for a patient. Because inhibition would be an undetectable source of false negativity, interpretation of

a negative AMPLICOR MTB Test result is to be considered presumptive for the presence of *M. tuberculosis* complex, pending culture results.

Since AFB smear positive specimens, will have a higher frequency of *M. tuberculosis* regardless of the prevalence of TB in the patient population, the predictive value of a positive AMPLICOR MTB Test result is high and provides clinically relevant information as an adjunct to AFB smear results. This result may assist in the earlier diagnosis of tuberculosis in individual patients.

IX. PANEL RECOMMENDATION

On January 25, 1996, the Microbiology Devices Panel voted to approve the AMPLICOR MTB Test with conditions. The conditions were:

A. That the AMPLICOR MTB Test be intended for use on AFB smear-positive samples only.

B. For discrepant samples (AFB smear positive/AMPLICOR MTB Test negative), both smear and AMPLICOR tests should be repeated in duplicate. The majority result for each test will be considered the correct result.

C. An optional spike back procedure should be included in the package insert for laboratories that wish to investigate the presence of inhibitions.

D. Additional language should be included in the package insert regarding AFB smear positive/AMPLICOR MTB Test negative samples, to include the likelihood that MOTT alone or MOTT plus *M. tuberculosis* complex may be present in the sample but undetectable due to the presence of inhibitors or the presence of low numbers of *M. tuberculosis* complex in the sample.

E. The manufacturer must expand the quality control section with material on how to recognize and trouble shoot assays that are not in control. The three negative controls should be interspersed throughout the run. Also, a lysis control utilizing an avirulent strain of *M. tuberculosis* (i.e., H37RA) that is spiked into a culture-negative, smear negative sample be developed and used daily.

F. Recommend post marketing monitoring of final culture results and test performance at each site during the first 6 months of use, and for the presence of inhibitors in samples at each site indirectly. Specific studies need only be done at sites where problems with inhibition are apparent

G. Further information to clarify test performance including reproducibility should be provided to FDA.

H. A table showing the effects of prevalence of tuberculosis on assay performance should be included in the package insert.

I. Training should be provided by the manufacturer for all new users that will permit the user to demonstrate their proficiency in performing the assay.

X. FDA DECISION

CDRH concurred with the recommendation of the Panel. CDRH issued an approvable letter to the applicant on March 13, 1996, requesting post-approval studies to:

a. monitor final culture results and AMPLICOR test performance at facilities using the AMPLICOR MTB Test,

b. assess the effects of inhibition on AMPLICOR MTB Test results,

c. to assess the results of replicate and repeat testing for AFB smear positive/AMPLICOR MTB Test negative specimens and the value of consensus results for these specimens (compared to concomitant culture and also to composite culture results for those patients with such specimens).

This monitoring would be performed at each site during the first 6 months of use; the number of sites to be monitored on a continuing basis would be assessed by FDA based on review of the monitoring results submitted in the first year following approval. Additional labeling modifications may be recommended after assessment of monitoring results.

An approval order was issued for the applicant's PMA for AMPLICOR® Mycobacterium Tuberculosis Test to Roche Molecular Systems on November 26, 1996 that specified postapproval studies to be completed.

The applicant's manufacturing and control facilities were inspected on November 21, 1995, and the facilities were found to be in compliance with the Good Manufacturing Practice Regulations (GMPs). Expiration dating for this device has been established and approved at twelve months, based on the most labile component, Mycobacteria Master Mix.

XI. APPROVAL SPECIFICATIONS

Directions for Use: See attached labeling

Conditions of Approval: CDRH approval of the PMA is subject to compliance with the conditions described in the approval order

XII. BIBLIOGRAPHY

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AMPLICOR® Mycobacterium Tuberculosis Test

FOR *IN VITRO* DIAGNOSTIC USE

Sputum Specimen Preparation Kit	83004	100 Tests
Mycobacterium Amplification Kit	83013	96 Tests
M. tuberculosis Detection Kit	83072	96 Tests

INTENDED USE

The AMPLICOR® Mycobacterium Tuberculosis (MTB) Test is a target amplified *in vitro* diagnostic test for the qualitative detection of *M. tuberculosis* complex DNA in concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens including bronchoalveolar lavages or aspirates, or tracheal aspirates.

The AMPLICOR MTB Test is intended for use as an adjunctive test for evaluating acid fast bacilli (AFB) smear positive sediments prepared using NALC-NaOH or NaOH digestion-decontamination of respiratory specimens from untreated patients suspected of having tuberculosis. Untreated patients are patients who have: (1) received no antituberculosis therapy, (2) had less than 7 days of therapy, or (3) have not received such therapy in the last twelve months. Only untreated patients may be evaluated with this test. The AMPLICOR MTB Test should only be performed in institutions proficient in the culture and identification of *M. tuberculosis* (ATS Level II and III[1] or CAP extent 3 and 4[2]). The test should always be performed in conjunction with mycobacterial culture.

WARNINGS

The efficacy of this test for the detection of *M. tuberculosis* from non-respiratory specimens, including but not limited to blood, CSF, stool or urine, has not been determined. The performance of the AMPLICOR MTB Test has not been validated for sediments processed by methods different than described or stored for time periods or at temperatures different than specified in this package insert.

AFB smear positive sediments must be cultured to: (1) determine if Mycobacteria other than *M. tuberculosis* (MOTT) are present, instead of/or in addition to MTB complex, (2) assess viability, (3) perform antimycobacterial drug susceptibility testing, or (4) determine the subspecies of *M. tuberculosis* complex (e.g., *M. bovis*) present.

AFB smear positive specimens may be AMPLICOR MTB Test negative and *M. tuberculosis* complex culture positive. This may be caused by: (1) the presence of low numbers of *M. tuberculosis* complex organisms in the presence of large numbers of MOTT, (2) inhibition of the AMPLICOR MTB Test, or (3) laboratory procedural errors.

Although specimens from pediatric patients, HIV positive patients, and patients with MOTT infections were tested during the clinical evaluations, total numbers were insufficient to definitively conclude that there were no statistical performance differences in these specific

patient populations.

The AMPLICOR MTB Test has not been evaluated with specimens from patients on antimycobacterial therapy to determine bacteriologic cure or to monitor response to such therapy.

PRECAUTIONS

A. For *in vitro* Diagnostic Use.

B. The AMPLICOR MTB Test is specific for, but does not differentiate among, members of the *M. tuberculosis* complex, i.e., *M. tuberculosis, M. bovis, M.bovis BCG, M. africanum,* and *M. microti.*

C. A negative test result does not exclude the possibility of isolating an *M. tuberculosis* complex organism from the specimen. Test results may be affected by specimen collection and transport, variability in specimen sampling, laboratory procedural errors, sample misidentification, and/or transcriptional errors.

D. This test is for use only in evaluating AFB smear positive sediments prepared using NALC-NaOH or NaOH digestion-decontamination as recommended by the Centers for Disease Control and Prevention (CDC)[3]. The effects of different specimen resuspension solutions has not been fully assessed. In the AMPLICOR Mycobacterium Tuberculosis Test clinical study, the following resuspension fluids were used: phosphate buffer (pH 6.8), BSA and BSA containing penicillin G and Le Motte's indicator. This test may only be used with concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens including bronchoalveolar lavages or aspirates, or tracheal aspirates.

E. Workflow in the laboratory must proceed in a uni-directional manner beginning in the Pre-Amplification Area and moving to the Post-Amplification (Amplification/Detection) Area. Pre-amplification activities should begin with reagent preparation and proceed to specimen preparation. Supplies and equipment must be dedicated to each pre-amplification activity and not used for other activities or moved between areas. Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-amplification supplies and equipment must remain in the Post-Amplification Area at all times. Plugged (aerosol barrier) or positive displacement tips must be used where specified.

F. Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories[4] and in the NCCLS Document M29-T[5]. All manipulations involving clinical specimens should be performed in a properly functioning biological safety cabinet using BioSafety Level 2 practices. Thoroughly clean and disinfect all work surfaces with 10% bleach. Autoclave any equipment or materials that have come in contact with clinical specimens before discarding.

G. Do not eat, drink or smoke in laboratory work areas. Wear disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.

H. Avoid microbial contamination of reagents when removing aliquots from reagent bottles. The use of sterile disposable pipettes and pipette tips is recommended.

I. Do not pool reagents from different lots or from different bottles of the same lot.

J. Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

K. Do not use a kit after its expiration date.

L. Material Safety Data Sheets (MSDS) are available on request from the Roche Response Center® or your local Roche office. See the telephone numbers listed on the last page of this insert.

M. Sputum Wash Solution, Sputum Lysis Reagent, Sputum Neutralization Reagent, *Mycobacterium* Master Mix, AmpErase®, *M. tuberculosis* (+) Control, and *Mycobacterium* (-) Control contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.

N. Wear eye protection, laboratory coats and disposable gloves when handling Denaturation Solution, Avidin-HRP Conjugate, Substrate A, Substrate B, and mixed Substrate A and Substrate B reagent (Working Substrate). Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. If spills of these reagents occur, dilute with water before wiping dry. Burns can occur if left untreated.

O. Avoid contact between the skin or mucous membranes and Substrate B or the Working Substrate. Wash immediately with large amounts of water if skin contact occurs.

P. Substrate B and Working Substrate contain dimethylformamide which is an irritant and has been reported to be teratogenic in high oral doses. Wear gloves when handling these reagents. Skin contact, inhalation of fumes and ingestion should be avoided.

SUMMARY AND EXPLANATION OF THE TEST

The AMPLICOR Mycobacterium Tuberculosis Test detects *M. tuberculosis* complex DNA in human respiratory specimens. Species of the *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum and M. microti)* are acid-fast, non-motile, rod-shaped, slow-growing aerobic bacteria that do not form spores. Both *M. tuberculosis* and *M. bovis* can cause tuberculosis, an infection often of lifelong duration that can result in disease in virtually every organ system, but most commonly in the lungs. Disease due to *M. bovis* is relatively uncommon. *M. africanum* has been shown to cause pulmonary disease in tropical areas and *M. microti* is not known to infect humans.

Worldwide, *M. tuberculosis* is the most common mycobacterial pathogen. It is found only in humans, who provide its reservoir and transmit the disease from person-to-person by droplet infection. Approximately one-third of the world's population harbors *M. tuberculosis* and is at risk for developing tuberculosis. In the United States, there are an estimated 10 million persons already infected with *M. tuberculosis* and over 20,000 cases of active tuberculosis disease are reported annually[6]. Due to the risk of the spread of the disease and the potential for the emergence of drug-resistant strains, the rapid detection of the *M. tuberculosis* complex is extremely important to establish a diagnosis of tuberculosis. Rapid detection of *M. tuberculosis* complex is critical in patients co-infected with HIV, as they are 30 times more likely to develop disease than a non-HIV infected person infected with *M. tuberculosis*[7]. The World Health Organization (WHO) estimates that 5.6 million people are co-infected with *M. tuberculosis* and HIV worldwide[8]. In global terms, tuberculosis accounts for 6.7% of all deaths in the developing world and 26% of avoidable adult deaths[8-12].

Laboratory identification of *M. tuberculosis* complex is required for the definitive diagnosis of tuberculosis. Routine cultures are time-consuming and can take up to eight weeks. Microscopic examination of acid-fast smears is the most rapid method for the detection of mycobacteria, but is insensitive and non-specific. Immunological and serological techniques are limited, in general, due to poor sensitivity and/or specificity[13,14]. The analysis of mycolic acids by high-performance liquid chromatography, biochemical testing and the testing of culture isolates with species-specific nucleic acid probes are used for species identification[15,16]. The development of nucleic acid amplification tests such as the AMPLICOR Mycobacterium Tuberculosis Test enables amplification and direct detection of mycobacterial DNA with specific nucleic acid probes prior to culture isolation and identification[17-22].

PRINCIPLES OF THE PROCEDURE

The AMPLICOR MTB Test is based on four major processes: specimen preparation; PCR amplification [23,24] of target DNA using biotinylated primers; hybridization of the amplified products to oligonucleotide probes specific to the target; and detection of the probe-bound amplified product by color formation.

Specimen Preparation

NALC/NaOH or NaOH liquefied, decontaminated and concentrated human respiratory specimens, including expectorated and induced sputum, bronchial specimens such as bronchoalveolar lavages or aspirates, or tracheal aspirates, are washed with Sputum Wash Solution. Organisms are lysed by incubation in Sputum Lysis Reagent and the specimen is made amplification ready by the addition of Sputum Neutralization Reagent.

Target Description

Selection of a target DNA sequence depends on identification of regions within the mycobacterial genome that show maximum sequence conservation among *M. tuberculosis* complex species. Accordingly, the appropriate selection of primers and probe is critical to the ability of the test to detect all organisms of the *M. tuberculosis* complex. The *Mycobacterium* genome contains a region of approximately 1500 nucleotides encoding the gene for 16s rRNA. The AMPLICOR MTB Test uses the *Mycobacterium* genus specific biotinylated primers KY18 and KY75 to define a sequence of approximately 584 nucleotides within this region[25,26].

PCR Amplification

The processed specimens are added to the Master Mix reagent which contains the thermal stable DNA polymerase, *Taq* polymerase, and heated. As the mixture cools, the biotinylated primers KY18 and KY75 anneal to the target DNA. *Taq* polymerase in the presence of excess deoxynucleoside triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine (in place of thymidine) triphosphates, extends the annealed primers along the target templates to produce a DNA sequence termed an amplicon. The process of heating and cooling is repeated for a number of cycles, each cycle effectively doubling the amount of amplicon DNA.

Selective Amplification

Selective amplification of target nucleic acid from the clinical specimen in the AMPLICOR MTB Test is achieved by the use of AmpErase. AmpErase (uracil-N-glycosylase, UNG) recognizes and catalyzes the destruction of DNA containing deoxyuridine, but not DNA containing thymidine[27]. Deoxyuridine is not present in naturally occuring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate in place of thymidine triphosphate as one of the dNTPs in the Master Mix reagent; thus only amplicon DNA contains deoxyuridine. The presence of deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase prior to amplification of the target DNA. AmpErase catalyzes the cleavage of deoxyuridine containing DNA at deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated to temperatures greater than 90°C in the first thermal cycling step at the alkaline pH of Master Mix, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. AmpErase is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. Following amplification, any residual enzyme is denatured by the addition of the Denaturation Solution, thereby preventing the degradation of target amplicon. AmpErase in the AMPLICOR MTB Test has been demonstrated to inactivate at least 10^3 copies/ reaction of deoxyuridine-containing MTB amplicon. This level of amplicon contamination is equivalent to that which may be caused by aerosolization. AmpErase may not prevent specimen contamination errors due to spills or splashes from a different, infected specimen.

Hybridization Reaction

Following PCR amplification, amplicon are chemically denatured to form single stranded DNA. Aliquots of this mixture are then added to a microwell plate (MWP) containing an oligonucleotide probe (KY172T3) specific for organisms of the *M. tuberculosis* complex. The biotin-labeled amplicon is captured by the probe-coated MWP. This hybridization of amplicon to the target-specific probe increases the overall specificity of the test.

Detection Reaction

The MWP is washed to remove unbound material and Avidin-Horseradish Peroxidase Conjugate (Av-HRP) is added. The Av-HRP binds to the biotin-labeled amplicon captured by the target specific DNA probe bound to the MWP. Unbound conjugate is removed by washing and substrate is added. The MWP-bound horseradish peroxidase catalyses the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide to form a colored complex. The reaction is stopped by the addition of weak acid. The intensity of the colored complex is measured by an automated microwell plate reader at a wavelength of 450 nm and the results are compared to the assay cut-off value.

REAGENTS

AMPLICOR Sputum Specimen Preparation Kit (83004)	100 tests
Sputum Wash Solution A Tris-HCl solution containing 1% solubilizer and 0.05% sodium azide (as a preservative)	2 x 25 mL

Sputum Lysis Reagent — 2 x 6 mL
A solution containing 1% solubilizer, 0.4% sodium hydroxide and 0.05% sodium azide (as a preservative)

Sputum Neutralization Reagent — 2 x 5 mL
A Tris-HCl solution containing 0.05% sodium azide (as a preservative)

AMPLICOR *Mycobacterium* Amplification Kit (83013) — 96 tests

Mycobacterium Master Mix — 3 x 1.5 mL
A Tris-HCl solution containing 20% glycerol, 0.05% sodium azide (as a preservative), <0.01% AmpliTaq® (*Taq* Polymerase), <0.001% dATP, dCTP, dGTP and dUTP, and < 0.005% biotinylated primers

AmpErase — 3 x 0.1 mL
A Tris-HCl solution containing <0.01% uracil-N-glycosylase, <1% EDTA, DTT, NaCl, solubilizer and 0.05% sodium azide (as a preservative)

M. tuberculosis (+) Control — 1 x 0.75 mL
Non-infectious *M. tuberculosis* DNA in a Tris-HCl, EDTA solution containing non-specific carrier DNA and 0.05% sodium azide (as a preservative)

Mycobacterium (-) Control — 1 x 0.75 mL
Non-specific non-infectious DNA in a Tris-HCl, EDTA solution containing non-specific carrier DNA and 0.05% sodium azide (as a preservative)

AMPLICOR *M. tuberculosis* Detection Kit (83072) — 96 Tests

Denaturation Solution (1) — 1 x 12 mL
A solution containing 3% EDTA, 1.6% sodium hydroxide and thymol blue

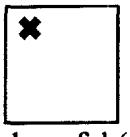
Xi  Contains 1.6% sodium hydroxide
irritant

Mycobacterium Hybridization Buffer (2) — 1 x 12 mL
A sodium phosphate and sodium salt solution containing <0.2% solubilizer and <25% chaotrope

Avidin-HRP Conjugate (3) — 1 x 12 mL
Avidin-horseradish peroxidase conjugate in a Tris-HCl solution containing 1% ProClin® 150 (as a preservative), emulsifier, bovine gamma globulin and 0.1% phenol

Substrate A (4A) — 1 x 12 mL
A citrate solution containing 0.01% H_2O_2 and 0.1 % ProClin® 150 (as a preservative)

Substrate B (4B) — 1 x 3 mL

Xn  Contains 0.1% 3,3',5,5'- tetramethylbenzidine (TMB) in 40% dimethylformamide

harmful (possible teratogen)

Stop Reagent (5) — 1 x 12 mL
A solution containing 4.9% sulfuric acid

10X-Wash Concentrate — 2 x 90 mL
A sodium phosphate and sodium salt solution containing EDTA, <2% detergent and 0.5% ProClin® 300 (as a preservative)

M. tuberculosis Microwell Plate — 1 x 96 tests
M. tuberculosis DNA Probe Coated Microwell Plate.
96 wells (Twelve, 8-well strips in one resealable pouch with desiccant)

STORAGE AND HANDLING REQUIREMENTS

A. Do not freeze reagents.

B. *Mycobacterium* Master Mix and AmpErase must be stored at 2-8°C. These reagents are stable until the expiration date indicated. Working Master Mix (Master Mix to which AmpErase has been added) must be stored at 2-8°C and is stable for 4 weeks.

C. *M. tuberculosis* (+) Control and *Mycobacterium* (-) Control must be stored at 2-8°C. These reagents are stable until the expiration date indicated.

D. Sputum Wash Solution, Sputum Lysis Reagent and Sputum Neutralization Reagent should be stored at 2-25°C. These reagents are stable until the expiration date indicated.

E. The *M. tuberculosis* DNA Probe Coated Microwell Plate (12 x 8-well strips) must be stored at 2-8°C in the foil pouch provided. The plate is stable in the unopened

pouch until the expiration date indicated. Once opened, the 8-well strips are stable for 3 months (or until the expiration date, whichever comes first).

F. Denaturation Solution, *Mycobacterium* Hybridization Buffer, and Stop Reagent should be stored at 2-25°C. These reagents are stable until the expiration date indicated.

G. Avidin-HRP Conjugate must be stored at 2-8°C. Unopened, this reagent is stable until the expiration date indicated. Once opened, this reagent is stable for 3 months (or until the expiration date, whichever comes first).

H. Substrate A and Substrate B must be stored at 2-8°C. These reagents are stable until the expiration dates indicated. Working Substrate must be prepared prior to use by mixing Substrate A with Substrate B. The Working Substrate is stable for 3 hours. Do not expose Substrate A, Substrate B or Working Substrate to metals, oxidizing agents or direct light.

I. 10X-Wash Concentrate should be stored at 2-25°C. The solution is stable until the expiration date indicated. Working Wash Buffer (1X) should be stored at 2-25°C in a clean, closed plastic container and is stable for 2 weeks from the date of preparation.

J. Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

MATERIALS PROVIDED:

AMPLICOR MTB Test

A. AMPLICOR Sputum Specimen Preparation Kit
Sputum Wash Solution
Sputum Lysis Reagent
Sputum Neutralization Reagent

B. AMPLICOR *Mycobacterium* Amplification Kit
Mycobacterium Master Mix
AmpErase
M. tuberculosis (+) Control
Mycobacterium (-) Control

C. AMPLICOR *M. tuberculosis* Detection Kit
Denaturation Solution
Mycobacterium Hybridization Buffer
Avidin-HRP Conjugate
Substrate A
Substrate B
Stop Reagent
10X-Wash Concentrate
M. tuberculosis DNA Probe Coated MWP

MATERIALS REQUIRED BUT NOT PROVIDED

Pre-Amplification - Reagent Preparation

Consumables (MicroAmp® Reaction Tubes, Caps, Base, Tray and Retainer) for either the Perkin-Elmer GeneAmp® PCR System 9600 or GeneAmp PCR System 2400 Thermal Cycler
- Eppendorf® Repeater® pipet and 1.25 mL individually wrapped Combitip® Reservoirs or equivalent
- Micropipettes with plugged (aerosol barrier) or positive displacement tips (100 µL)*
- Plastic resealable bags
- Latex gloves

Pre-Amplification - Specimen Preparation

- Microcentrifuge (max. RCF 16,000 x g, min. RCF 12,500 x g); Eppendorf 5415C, HERMLE Z230M, or equivalent
- Sterile distilled water
- #1 McFarland Turbidity Nephelometer Standard
- *M. tuberculosis* Strain (avirulent strain), ATCC #25177
- 15 mL sterile conical centrifuge tubes (Corning 25319-15 or equivalent)
- 1.5 mL polypropylene screw cap tubes, sterile (Sarstedt 72.692.105 or equivalent)
- Tube racks (Sarstedt 93.1428)
- Sterile fine tip transfer pipettes (Fisher 13-711-28 or equivalent)
- Vortex mixer with variable speed
- Micropipettes with plugged (aerosol barrier) or positive displacement tips (100 µL, 200 µL and 1000 µL)*
- 60°C ± 2°C dry heat block (containing 0.5 cm sand)
- MicroAmp 2400 Base or MicroAmp 9600 Base
- MicroAmp Cap Installing Tool

Post Amplification Area - Amplification/Detection

- Perkin-Elmer GeneAmp PCR System 9600 or GeneAmp PCR System 2400 Thermal Cycler
- Multi-channel pipettor (25 μL and 100 μL)
- Plugged (aerosol barrier) micropipette tips (200 μL) and unplugged tips (200 μL)*
- Disposable reagent reservoirs
- Microwell plate lid
- Elisawell key for strip removal
- 37°C ± 2°C Incubator
- Distilled or deionized water
- Graduated cylinder
- Microwell plate washer** (recommended but not required)
- Microwell plate reader capable of reading 450 nm and printer***
- Latex gloves

* Micropipettes should be accurate within 3% of stated volume. Plugged (aerosol barrier) or positive displacement tips must be used where specified.

** Capable of washing 12 x 8 microwell format with 350-450 μL of Wash Solution per well at 30 second (± 10 seconds) timed intervals.

*** Microwell Plate Reader Specifications: Bandwidth = 10 ± 3 nm; Absorbance Range: 0 to ≥ 3.00 A_{450}; Repeatability ≤ 1%; Accuracy ≤ 3% from 0 to 3.00 A_{450}; Drift ≤ 0.01 A_{450} per hour.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Handle all specimens as if they are capable of transmitting infectious agents.

A. Specimen Collection And Storage

Specimens must be collected in sterile plastic containers and stored at 2-8°C if the transport to the laboratory will be delayed by more than 1 hour. Specimens should be processed (decontaminated and concentrated) within 24 hours of collection (including transport time) as recommended by the CDC[3].

B. Specimen Transport

Transport freshly collected specimens to the laboratory as soon as possible. Specimens must be shipped in compliance with country, federal, state and local regulations for the transport of etiologic agents[28, 29].

C. Specimen Processing

The AMPLICOR MTB Test is designed to detect DNA from the *M. tuberculosis* complex using sediments prepared from respiratory specimens which have been processed from accepted adaptations of the NALC/NaOH or NaOH decontamination procedures described by the CDC[3] using 2% to 3% NaOH for 15-20 minutes and centrifugation at $\geq$ 3,000 x g.

D. Processed Sediment Storage

Decontaminated and concentrated sediments may be stored at 2-8°C for up to 4 days. The processed sediments are stable for up to 6 months when stored at -70°C.

E. Amplification Ready Lysates

Lysates which have been prepared by the AMPLICOR MTB Test specimen preparation procedure (amplification ready lysates) may be stored at 2-8°C for up to 4 days. The amplification ready lysates are stable for up to 6 months when stored at -70°C.

PREPARATION OF SPECIMEN LYSIS CONTROL

It is recommended that a Specimen Lysis Control be included in each test run to control for the specimen lysis step of the AMPLICOR MTB Test procedure. The organisms used for this control should be an avirulent *M. tuberculosis* strain (e.g., ATCC #25177). The *M. tuberculosis* Specimen Lysis Control should be prepared as indicated in the following instructions. The Specimen Lysis Control should be prepared in advance, divided into 100 µL aliquots, frozen at -20°C (for up to 6 months) or at -70°C (for up to 12 months), and thawed as needed for testing.

1. Place 3 to 5 sterile 3 mm glass beads in a sterile culture tube.

2. Add 1-2 mL sterile water to each tube. Add the avirulent ATCC strain organisms from a culture to the tube. Cap and repeatedly vortex the tube to produce a homogeneous solution.

3. Allow the bacterial suspension to settle for 15 minutes.

4. Transfer the supernatant to a sterile, transparent culture tube. Adjust the turbidity to a #1 McFarland nephelometer standard. This stock should contain approximately 10^8 cells/mL.

5. Make a 1:25 dilution (Dilution A) of the cell suspension by adding 50 µL of the #1 McFarland stock to 1.2 mL of liquefied, decontaminated and concentrated negative sputum. Cap the tube and vortex. (NOTE: The sputum used for this and subsequent dilutions should be known to be culture negative for mycobacterium species).

6. Make a 1:10 dilution (Dilution B) of the cell suspension by adding 100 µL of Dilution A to 900 µL of decontaminated negative sputum. Cap the tube and vortex. If desired, a larger volume of Dilution B may be prepared by proportionally increasing the amount of Dilution A and decontaminated negative sputum.

7. Make a second 1:10 dilution (Dilution C) of the cell suspension by adding 100 µL of Dilution B to of 900 µL decontaminated negative sputum. Cap the tube and vortex. If desired, a larger volume of Dilution C may be prepared by proportionally increasing the amount of Dilution B and decontaminated negative sputum.

8. Make a third 1:10 dilution (Dilution D) of the cell suspension by adding 100 µL of Dilution C to 900 µL of decontaminated negative sputum. Cap the tube and vortex. If desired, a larger volume of Dilution D may be prepared by proportionally increasing the amount of Dilution C and decontaminated negative sputum.

9. Make a fourth 1:10 dilution (Dilution E) of the cell suspension by adding 100 µL of Dilution D to 900 µL of decontaminated negative sputum. Cap the tube and vortex. If desired, a larger volume of Dilution E may be prepared by proportionally increasing the amount of Dilution D and decontaminated negative sputum.

10. Assay 100 µL aliquots of Dilution B, C, D and E using the AMPLICOR MTB Test. Store the remainder of each dilution for up to 24 hours at 2-8°C until Steps 11 and 12 are completed.

11. Select the dilution (B, C, D or E) that consistently gives AMPLICOR Test results of 3.0 A_{450} or greater and where the next lower dilution (C, D or E) gives results less than 3.0 A_{450}.

12. Pipet 100 µL single use aliquots of the selected dilution into sterile 1.5 mL specimen preparation tubes. Store the tubes frozen at -20°C (for up to 6 months) or at -70°C (for up to 12 months). Frost-free freezers should <u>not</u> be used.

13. Thaw and test one aliquot of the Specimen Lysis Control according to the Instructions for Use, Part B, "Specimen Preparation", Steps 3-11, each time specimen preparation is performed.

INSTRUCTIONS FOR USE

NOTE: If specimen processing, amplification and detection are performed in a single work day, follow the test procedure as described below. If specimen preparation is performed on a separate day from amplification and detection, perform Reagent Preparation (Part A) on the same day that Amplification and Detection (Part C) are to be done.

A. REAGENT PREPARATION

Performed in: Pre-Amplification - Reagent Preparation Area

1. Determine the appropriate number of MicroAmp Reaction Tubes needed for patient specimen and control testing. It is recommended that one replicate of the *M. tuberculosis* (+) Control, three replicates of the *Mycobacterium* (-) Control, and one replicate of the Specimen Lysis Control be included in each AMPLICOR run. Place tubes in MicroAmp Tray and lock in place with Retainer.

2. Prepare Working Master Mix by adding 100 µL of AmpErase to 1 vial of *Mycobacterium* Master Mix (the mixture is sufficient for 32 amplifications). Recap the Master Mix vial and mix well by inverting 10-15 times. Record the date of preparation on the vial. Working Master Mix (Master Mix to which AmpErase has been added) is stable at 2-8°C for 4 weeks. Discard the empty AmpErase vial.

3. Pipet 50 µL of Working Master Mix into each MicroAmp Reaction Tube using a Repeater Pipet and 1.25 mL Combitip Reservoir or a micropipette with a plugged (aerosol barrier) tip.

4. Place the MicroAmp Tray containing Working Master Mix in a resealable plastic bag. Seal the plastic bag securely and move the MicroAmp Tray to the Pre-Amplification-Specimen Preparation Area. Store the MicroAmp Tray containing Working Master Mix at 2-8°C in the Pre-Amplification - Specimen Preparation Area until ready for use.

B. SPECIMEN AND CONTROL PREPARATION

Performed in: Pre-Amplification - Specimen Preparation Area

1. Determine the number of samples to be tested and arrange sufficient 1.5 mL screw-cap tubes in the work area to have one tube for each specimen and one tube for each control (*M. tuberculosis* (+) Control, *Mycobacterium* (-) Control, and Specimen Lysis Control). Label each specimen preparation tube with the specimen identification number.

2. Add 100 µL of each liquefied, decontaminated and concentrated respiratory specimen and the lysis control to 500 µL of Sputum Wash Solution in a 1.5 mL screw-cap tube. Vortex.

3. Centrifuge at a minimum of 12,500 x g for 10 minutes.

4. Aspirate supernatant using a fine tip transfer pipette and add 100 µL of Sputum Lysis Reagent to the cell pellet. Vortex to resuspend the pellet.

5. Prepare the *M. tuberculosis* (+) and *Mycobacterium* (-) Working Controls.

   Note: Working Controls must be prepared fresh each day the test is performed. Working Controls can be used to prepare multiple Processed Controls during the day, but must be discarded at the end of the day.

   (a) Vortex the *M. tuberculosis* (+) Control vial for 5 seconds. Pipette 100 µL of *M. tuberculosis* (+) Control into a 1.5 mL screw-cap tube using a micropipette with a plugged tip. Add 400 µL of Sputum Lysis Reagent. Vortex. This is the *M. tuberculosis* (+) Working Control. Store at 2-8°C and discard at the end of the work day

   (b) Vortex the *Mycobacterium* (-) Control vial for 5 seconds. Pipette 100 µL of *Mycobacterium* (-) Control into a 1.5 mL screw-cap tube using a micropipette with a plugged tip. Add 400 µL of Sputum Lysis Reagent. Vortex. This is the *Mycobacterium* (-) Working Control. Store at 2-8°C and discard at the end of the work day

   (c) Pipette 100 µL from each Working Control into a 1.5 mL screw-cap tube to be processed in parallel with the clinical specimens and the Specimen Lysis Control.

6. Incubate washed specimens and controls in a 60°C ± 2°C dry heat block (containing 0.5 cm sand) for 45 minutes.

7. Remove tubes from the heat block and pulse centrifuge the tubes for 5 seconds to remove condensate from the cap.

8. Add 100 µL of Sputum Neutralization Reagent to each specimen and control tube. Vortex for 5 seconds at half speed.

9. Pipette 50 µL of each prepared patient specimen, the processed *M. tuberculosis* (+) Control, the processed *Mycobacterium* (-) Control, and the processed Specimen Lysis Control to the appropriate tubes using a micropipette with plugged tip(s). Use a new pipette tip for each specimen and control. Be careful of avoid transferring any material that may not have been resuspended. Record the positions of the patient specimens and controls on a tray map. Cap the tubes. Apply pressure for a tight seal using the GeneAmp PCR System cap installing tool.

10. Move the prepared samples (patient specimens and controls) in the MicroAmp Tray/Retainer Assembly to the Post-Amplification Area.

C. AMPLIFICATION AND DETECTION
Performed in: Post-Amplification - Amplification/Detection Area

C1. AMPLIFICATION

NOTE: Turn on the thermal cycler at least 30 minutes prior to beginning amplification.

1. Place the MicroAmp Tray/Retainer Assembly (without the MicroAmp Base) into the thermal cycler sample block.

2. Program the GeneAmp PCR System 9600 or GeneAmp PCR System 2400 Thermal Cycler as follows:

HOLD Program:	2 min at 50°C
CYCLE Program (2 cycles):	20 sec at 98°C, 20 sec at 62°C, 45 sec at 72°C
CYCLE Program (35 cycles):	20 sec at 94°C, 20 sec at 62°C, 45 sec at 72°C
HOLD Program:	5 min at 72°C
HOLD Program:	72°C FOREVER (≤ 18 hours)

In the CYCLE programs, the ramp times and the allowed set-point error should be left at the default settings of 0:00 (which is the maximum rate) and 2°C, respectively. Link the 5 programs together into a METHOD program. Consult either the GeneAmp PCR System 9600 or GeneAmp PCR System 2400 User's Manual for additional information on programming and operation of the thermal cycler.

3. Start the METHOD program. The program runs approximately 1.5 hours.

4. Remove the tray from the thermal cycler at any time during the final HOLD program, place in the MicroAmp Base and continue immediately with Step 5. Do not allow the reaction tubes to remain in the thermal cycler beyond the final HOLD program and do not extend the final HOLD program beyond 18 hours. DO NOT BRING AMPLIFIED SAMPLES INTO THE PRE-AMPLIFICATION AREA. AMPLIFIED CONTROLS AND SPECIMENS SHOULD BE CONSIDERED A SIGNIFICANT POTENTIAL SOURCE OF CONTAMINATION.

5. Remove the caps from the reaction tubes carefully, one row at a time, to avoid creating aerosols of the amplification products. Immediately pipette 100 μL of Denaturation Solution (1) to the first column (or row) of reaction tubes using a multichannel pipettor with plugged (aerosol barrier) tips and mix by pipetting up and down. For each column (or row), repeat this procedure using a fresh set of tips.

6. The denatured amplicon can be held at room temperature for no more than 2 hours before proceeding to the detection reaction. If the detection reaction can not be performed within 2 hours, re-cap the tubes using new caps and store the denatured amplicon at 2-8°C for up to 1 week.

C2. DETECTION

1. Warm all reagents to room temperature prior to use.

2. Prepare Working Wash Solution (1X) as follows. Examine the 10X-Wash Concentrate and, if necessary, warm at 30-37°C to redissolve any precipitate. Add 1 volume of 10X-Wash Concentrate to 9 volumes of distilled or deionized water. Mix well. For manual washing, prepare 40 mL of Working Wash Buffer (1X) for each 8-well MWP strip. For automated washing, the volume of Working Wash Solution required depends on the model of MWP washer being used. Store Working Wash Solution in a clean, closed plastic container at 2-25°C for up to 2 weeks.

3. Allow MTB MWP to warm to room temperature before removing from the foil pouch. Remove the appropriate number of 8-well MWP strips from the foil package and set into the MWP frame. Return unused strips to the pouch and reseal making sure that the desiccant pillow remains in the pouch. NOTE: MWP strips must be handled carefully to avoid breakage. To remove strips from the frame, center the MWP on top of the Elisawell key and press down evenly on the corners of the frame. To lock the strips in place, place the Elisawell key on top of the strips and press uniformly against the strips.

4. Add 100 μL of *Mycobacterium* Hybridization Buffer (2) to each well to be tested on the MWP.

5. If the denatured amplicon was stored at 2-8°C, incubate at 37°C for 2-4 minutes in order to reduce viscosity.

6. Using a multichannel pipettor with plugged (aerosol barrier) tips, pipette 25 μL of each denatured amplicon to the appropriate wells of the MWP. Gently tap the plate 10-15 times until the color changes from blue to light yellow; this color change indicates sufficient mixing has occurred.

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7. Cover the plate with a MWP lid and incubate for 1.5 hours at 37°C ± 2°C.

8. Wash the MWP 5 times either manually or by using an automated MWP washer using the Working Wash Solution.

   For manual washing:
   (a) Empty contents of plate and tap dry on paper towels.
   (b) Pipette Working Wash Solution to fill each well to top (400-450 µL). Let soak for 30 seconds. Empty out contents and tap dry.
   (c) Repeat Step (b) four additional times.

   For automated washing, program washer to:
   (a) Aspirate contents of wells.
   (b) Fill each well to top with Working Wash Solution (approximately 350-450 µL depending on plate washer), soak for 30 seconds and aspirate dry.
   (c) Repeat Step (b) four additional times.
   (d) Tap the plate dry.

9. Add 100 µL of Avidin-HRP Conjugate (3) to each well. Cover the MWP and incubate for 15 minutes at 37°C ± 2°C.

10. Wash MWP as described in Step 8.

11. Prepare Working Substrate by mixing 2.0 mL of Substrate A (4A) and 0.5 mL of Substrate B (4B) for each multiple of two, 8-well MWP strips (16 tests). Prepare this reagent no more than 3 hours before use. Store at room temperature and protect from exposure to direct light.

12. Pipette 100 µL of prepared Working Substrate into each well being tested.

13. Allow color to develop for 10 minutes at room temperature (20-25°C) in the dark.

14. Add 100 µL of Stop Reagent (5) to each well

15. Measure the optical density at 450 nm within 30 minutes of Stop Reagent addition. Record the absorbance value for each patient specimen and control tested.

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PROCEDURAL PRECAUTIONS

1. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Pre-Amplification Area and moving to the Post-Amplification (Amplification/Detection) Area. Pre-amplification activities must begin with reagent preparation and proceed to specimen preparation. Supplies and equipment must be dedicated to each pre-amplification activity and not used for other activities or moved between areas. Equipment and supplies used for reagent preparation must not be used for sample preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-amplification supplies and equipment must remain in the Post-Amplification Area at all times.

2. As with any test procedure, good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored for purity. Discard any reagents that may be suspect.

3. All pipettors, pipettes, bulbs, pipette tips, etc., should be dedicated to, and used only for, each AMPLICOR MTB laboratory activity.

QUALITY CONTROL

It is recommended that each AMPLICOR MTB run include at least one replicate of the Specimen Lysis Control, one replicate of the *M. tuberculosis* (+) control, and three replicates of the *Mycobacterium* (-) control. The three replicates of the Mycobacterium (-) Control should be interspersed between specimens throughout the tray. As with any new laboratory procedure, new operators should consider the use of additional positive and negative controls each time the test is performed until such time that a high degree of confidence is reached in their ability to perform the test procedure correctly.

The *M. tuberculosis* (+) Control will assure that critical reagents have been added and are functioning. The *Mycobacterium* (-) Control will assure that reagents or testing areas have not been contaminated and that the MWP washing has been performed. The Lysis Control will monitor functioning of the specimen lysis procedure. Use of the inhibition procedure is recommended as an additional control for detecting the presence of inhibitory substances in specimens which are negative by the AMPLICOR MTB test.

RESULTS

Controls

Each laboratory should establish target values and acceptable limits for the Specimen Lysis, *Mycobacterium* (-)Control and *M. tuberculosis* (+) Control using statistically derived rules as defined in the NCCLS C-24A Guideline. Table 1 shows the minimal acceptable A_{450} results for these controls:

Table 1
Minimum Acceptable A_{450} Results for Controls

Specimen Lysis Control	1.0 A_{450}
Mycobacterium (-) Control	0.25 A_{450} (all reps)
M. tuberculosis (+) Control	2.0 A_{450}

If these limits (or internally established limits) are not met, the run should be invalidated and the entire test procedure (specimen preparation, amplification and detection) should be repeated and patient specimen results should not be reported. If control values consistently do not meet the established limits, contact the Roche Response Center (or your local Roche office) for technical assistance. See the telephone numbers on the last page of this package insert.

Patient Specimens

The presence of *M. tuberculosis* complex DNA is determined by the A_{450} reading for each specimen as shown in Table 2.

Table 2
Interpretation of Test Results

A_{450} Result	AMPLICOR MTB Test Result Interpretation
0.2 to 0.6	Equivocal result, repeat testing on the specimen in duplicate. If either or both repeat results are again equivocal, the results are inconclusive and should not be reported. If both repeat results are < 0.2 A_{450} or if both repeat results are > 0.6 A_{450}, report the results as indicated below.
< 0.2	Specimen is a presumptive negative for *M. tuberculosis* complex DNA
> 0.6	Specimen is positive for *M. tuberculosis* complex DNA

In clinical studies, the majority of culture negative specimens were below 0.2 A_{450} and the majority of culture positive results were greater than 0.6 A_{450} (see Table 5 in the "Expected Values" section of this insert). Patient specimens with test results between 0.2 A_{450} and 0.6 A_{450} should be retested in duplicate and the final test result for the specimen reported as indicated in Table 2. Any specimen that is retested because the initial test result falls within

the range of 0.2 A_{450} to 0.6 A_{450} should also be tested for inhibition as described in the "Procedure for Assessing Specimen Inhibition" section of this insert.

INTERPRETATION AND REPORTING TEST RESULTS

1. If the AFB smear and AMPLICOR MTB Test are both positive, report the following:

   "AFB smear positive and *Mycobacterium tuberculosis* complex DNA detected. AFB culture pending. Specimen may contain either *M. tuberculosis* alone or both MOTT and *M. tuberculosis*"

2. If the AFB smear is positive and the AMPLICOR MTB Test is negative, report the following:

   "AFB smear positive, no *Mycobacterium tuberculosis* complex DNA detected. AFB culture pending. Specimen may not contain *M. tuberculosis*, or the AMPLICOR MTB Test result may be falsely negative due to low numbers of *M. tuberculosis* in the presence or absence of MOTT, or due to interference by inhibitors present in the specimen".

PROCEDURE FOR ASSESSING SPECIMEN INHIBITION

To assess if specimen inhibition could be the cause of AFB smear positive/AMPLICOR MTB negative test results, the following procedure should be used.

1. Prepare amplificaton reagents as indicated in Part A, "*Reagent Preparation*", in the Instructions for Use Section of the AMPLICOR MTB package insert.

2. Label two 1.5 mL screw-capped specimen preparation tubes for each specimen to be tested for inhibition. Label both tubes with the specimen identification number. Designate one tube as "unspiked" (U) and the other as "spiked" (S).

3. Add 500 μL of Sputum Wash Solution to each tube.

4. Add 100 μL of liquefied, decontaminated and concentrated respiratory specimen to each tube. Vortex to mix.

5. Follow Steps 3-5 in Part B, "*Specimen and Control Preparation*", of the Instructions for Use section of the package insert.

6. To the "unspiked" (U) tube add 100 μL of Sputum Lysis Reagent. Vortex to resuspend the cell pellet.

7. To the "spiked" (S) tube add 100 μL of *M. tuberculosis* (+) Working Control. Vortex to resuspend the cell pellet.

8. Continue testing starting with Step 6 in Part B, "*Specimen and Control Preparation*", of the Instructions for Use section of the package insert.

9. For a valid run, specimen results are interpreted as shown in Table 3.

Table 3
Interpretation of Inhhibition Assessment Test Results

Unspiked Tube A_{450} Reading	Spiked Tube A_{450} Reading	Inhibition Assessment Test Result Interpretation
0.6	0.6	Specimen is not inhibitory and is positive for *M. tuberculosis* complex DNA. Difference between original and repeat result is likely due to random sampling bias.
< 0.35	0.6	Specimen is not inhibitory and is a presumptive negative for *M. tuberculosis* complex DNA.
< 0.35	< 0.35	Specimen is inhibitory. A new specimen should be obtained for testing.

PROCEDURAL LIMITATIONS

1. This test is indicated for use only with concentrated sediments prepared from sputum (induced or expectorated), bronchial specimens including bronchoalveolar lavages or aspirates, or tracheal aspirates. Testing of other specimen types may result in false negative or false positive results.

2. Reliable results are dependent on adequate specimen collection and proper specimen transport and storage procedures.

3. Detection of *M. tuberculosis* is dependent on the number of organisms present in the specimen. This may be affected by specimen collection methods and patient factors such as age, presence of symptoms, and prior therapy.

4. Therapeutic success or failure cannot be determined using the AMPLICOR MTB Test as mycobacterial nucleic acid may be detected following appropriate chemotherapy.

5. The addition of AmpErase (UNG) to Master Mix enables selective amplification of target DNA. However, reagent purity is maintained only by good laboratory practices and careful adherence to the procedures specified in this insert. The UNG enzyme in the AMPLICOR Mycobacterium Tuberculosis Test has been shown to prevent false positive results due to contamination of up to 10^3 amplicon copies/reaction.

6. As with any diagnostic test, results from the AMPLICOR MTB Test should be interpreted with consideration of all clinical and laboratory findings.

7. Use of this product should be limited to personnel trained in the techniques of PCR.

8. Interfering Substances: White blood cells at concentrations in excess of 1 x 10^7 cells/mL may interfere with the AMPLICOR MTB Test. The bronchial dilators Primatene®, Proventil® and Vanceril® were tested at ten times the normal dosage and found not to interfere with the AMPLICOR MTB Test. Sputum Induction Solution (3% NaCl) was tested and found not to interfere with the AMPLICOR MTB Test.

9. Co-infection with Closely Related Organisms: The AMPLICOR MTB Test is specific for the detection of the MTB complex. However, because the nucleic acid primers used in the amplification reaction are genus specific, excessive numbers of MOTT ($\geq 10^5$ CFU/mL) or other species of organisms closely related to Mycobacteria such as *Rhodococcus, Corynebacterium* or *Gordonae* ($\geq 10^7$ CFU/mL) may cause false negative results due to the competitive amplification of these other organisms when low levels of *M. tuberculosis* are present in the specimen

EXPECTED VALUES

A. Range of Control Results Observed in the Clinical Studies

The mean and range of the A_{450} results observed in a ten site clinical study for the *M. tuberculosis* (+) Control and the *Mycobacterium* (-) Control are shown in Table 4. Two hundred sixty four (264) AMPLICOR Mycobacterium Tuberculosis Test runs were performed during the clinical study. Nine of the test runs were invalidated due to out of range control results; eight due to low positive control results, and one due to both high negative control results and low positive control results.

Table 4
Control Results from Clinical Study

	A_{450} Results (n=264)	
	Mean	Range
M. tuberculosis (+) Control	3.452	0.101 - 4.0*
Mycobacterium (-) Control	0.072	0.006 - 0.764**

* Nine results observed between 0.01 to 0.650 A_{450}; all others ranged from 2.009 to 4.0.
** One result observed greater than 0.20 A_{450}; all others ranged from 0.006 to 0.178

B. Distribution of Patient Specimen Results Observed in the Clinical Study

The range of the A_{450} results observed for the 189 smear positive patient specimens included in the AMPLICOR Mycobacterium Tuberculosis Test clinical study are shown in Figure 1 and Table 5

Figure 1

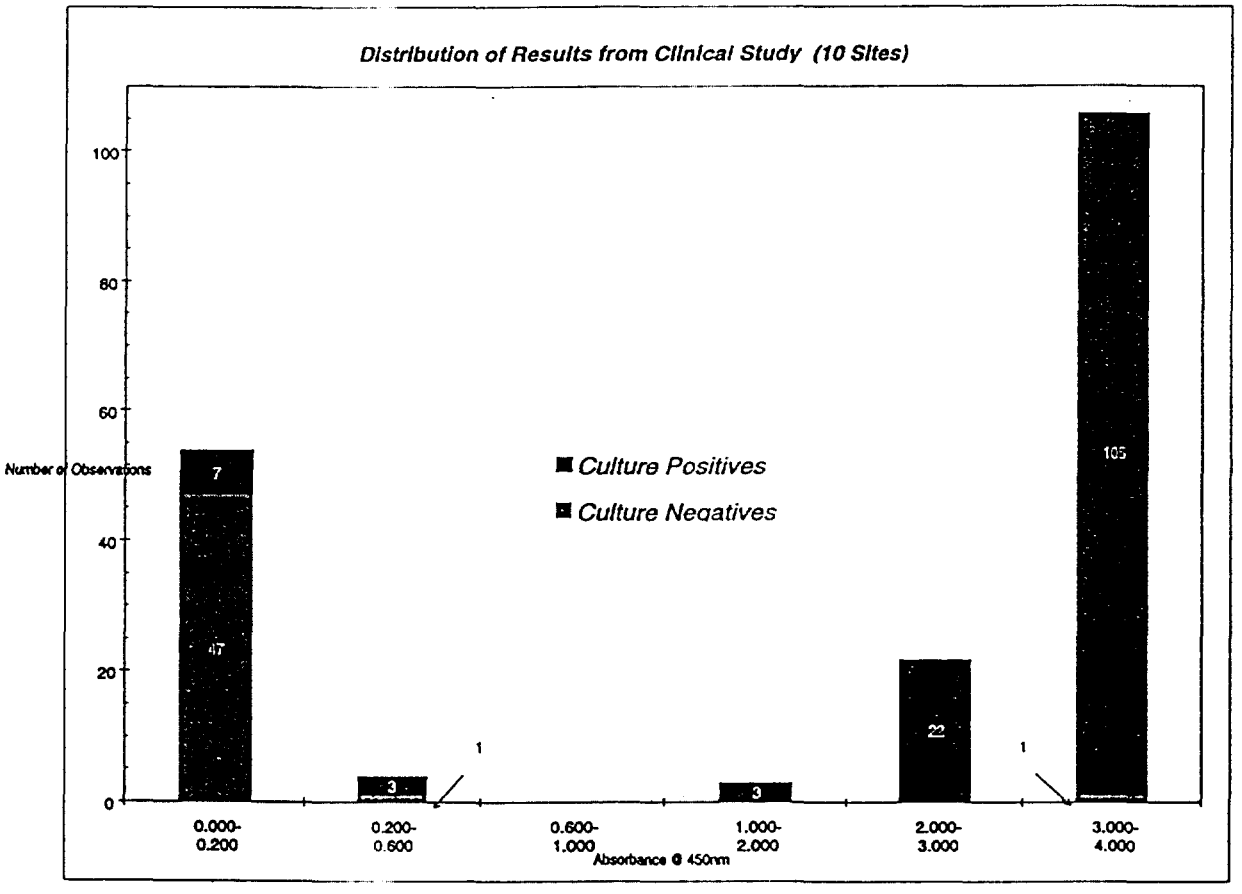

Table 5
Distribution of Patient Specimen Results from Clinical Study

	Absorbance Range (450 nm)/Number of AMPLICOR Results						
Range A_{450}	0.0 - 0.2	0.2 - 0.6	0.6 - 1.0	1.0 - 2.0	2.0 - 3.0	3.0 - 4.0	Total
No. Culture Positive	7	3	0	3	22	105	140
No. Culture Negative	47	1	0	0	0	1	49

PERFORMANCE CHARACTERISTICS

A. Limit of Detection

Thirty four (34) isolates of *M. tuberculosis*, including drug sensitive, drug resistant and multiple drug resistant strains, were obtained from diverse geographical regions in North America and tested by the AMPLICOR MTB Test. The AMPLICOR MTB Test reproducibly detected the presence *M. tuberculosis* at levels of 800 CFU/mL or greater, equivalent to ≥ 20 *M. tuberculosis* cells/reaction. The AMPLICOR MTB Test has been shown to detect 5 or more copies of purified *M. tuberculosis* DNA (as determined by Poisson analysis) in an aqueous sample matrix 100% of the time.

B. Analytical Specificity

The analytical specificity of the AMPLICOR MTB Test was evaluated by testing the following bacteria and viruses, many of which are normal flora or pathogens common to the respiratory tract. All bacterial isolates were tested using 10^7 cells/mL or the equivalent of 10^6 copies of *M. tuberculosis* nucleic acid/reaction. The viral isolates were tested at the PFU/mL levels indicated below. None of the isolates tested were positive by the AMPLICOR MTB Test.

Mycobacterial Species:

Mycobacterium asiaticum	*Mycobacterium leprae*
Mycobacterium aurum	*Mycobacterium malmoense*
Mycobacterium avium	*Mycobacterium marinum*
Mycobacterium celatum	*Mycobacterium neoaurum*
Mycobacterium chitae	*Mycobacterium nonchromogenicum*
Mycobacterium cookii	*Mycobacterium phlei*
Mycobacterium fallax	*Mycobacterium scrofulaceum*
Mycobacterium flavescens	*Mycobacterium smegmatis*
Mycobacterium fortuitum	*Mycobacterium sphagni*
Mycobacterium gastri	*Mycobacterium szulgai*
Mycobacterium genavense	*Mycobacterium terrae*
Mycobacterium gordonae	*Mycobacterium terrae-like strains*
Mycobacterium intracellulare	*Mycobacterium thermoresistibile*
Mycobacterium kansasii	*Mycobacterium triviale*
Mycobacterium komassense	*Mycobacterium xenopi*

Non-Mycobacterial Species:

Acinetobacter calcoaceticus
Actinomadura madurae
Actinomyces pyogenes
Actinoplanes italicus
Aeromonas hydrophila
Arcanobacterium haemolyticum
Arthrobacter oxydans
Bacillus subtilis
Bacteriodes fragilis
Blastomyces dermatitidis
Bordetella parapertussis
Bordetella pertussis
Branhamella (Moraxella) catarrhalis
Brevibacterium linens
Campylobacter jejuni
Candida albicans
Chlamydia pneumonia
Chlamydia trachomatis
Chromobacterium violaceum
Citrobacter freundii
Clostridium perfringens
Coccidioides immitis
Corynebacterium aquaticum
Corynebacterium diphtheriae
Corynebacterium flavescens
Corynebacterium glutamicum
Corynebacterium jeikeium
Corynebacterium minutissimum
Corynebacterium pseudodiphtheriticum
Corynebacterium pseudotuberculosis
Corynebacterium renale
Corynebacterium striatum
Corynebacterium xerosis
Cryptococcus neoformans
Deinococcus radiodurans
Dermatophilus congolensis
Derxia gummosa
Eikenella corrodens
Enterobacter cloacae
Enterococcus faecalis
Enterococcus faecium
Escherichia coli
Fusobacterium nucleatum
Gordona sputi
Haemophilus influenza
Haemophilus parainfluenzae
Histoplasma capsulatum
Klebsiella pneumoniae
Klebsiella pneumoniae subsp. ozaenae
Lactobacillus casei
Legionella micdadei
Legionella pneumophila
Microbacterium lactamica
Mycoplasma hominis
Mycoplasma pneumoniae
Neisseria gonorrhoeae
Neisseria lactamica
Neisseria meningitidis
Nocardia asteroides
Nocardia brasiliensis
Nocardia farcinica
Nocardia nova
Nocardia otitidiscaviarum
Nocardia transvalensi
Oerskovia turbata
Peptococcus niger
Peptostreptococcus anaerobius
Peptostreptococcus magnus
Pneumocycstis carinii
Porphyromonas asaccharolytica
Porphyromonas gingivalis
Prevotella melaninogenica
Propionibacterium acnes
Proteus mirabilis
Pseudomonas aeruginosa
Rhodococcus aichiensis
Rhodococcus bronchialis
Rhodococcus chubuensis
Rhodococcus equi
Salmonella choleraesuis subsp. choleraesuis
Serratia marcescens
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus pneumoniae
Streptococcus pyogenes
Streptomyces griseinus
Veillonella atypica
Veillonella parvula
Vibrio parahaemolyticus
Xanthomonas maltophilia
Yersinia enterocolitica

Viral Isolates:

VIRUS	*PFU/PCR Tested*
Adenovirus	3.2×10^5
Cytomegalovirus	9.8×10^1
Enterovirus	5.6×10^3
Herpes Simplex I	5.6×10^5
Influenza B	Unknown
Parainfluenza 2	2.8×10^5
Respiratory Syncytial Virus	2.8×10^1
Rhinovirus 14	2.8×10^4

C. Precision

The Between-Day, Between-Run, Within-Run and Total Precision of the AMPLICOR MTB Test were determined by analysis of dilutions of *M. tuberculosis* cells. The samples used for this study were simulated specimens prepared by spiking decontaminated sputum with *M. tuberculosis* (ATCC# 25177) The spiked specimens were quantitated by culture and found to contain approximately 0, 450, 950, and 1300 CFU/mL. Testing was performed at 4 sites, twice per day for 3 consecutive work days. Calculations were performed in accordance with the NCCLS EP5T guideline. The results of this study are summarized in Table 6.

Table 6
AMPLICOR MTB Test Precision

SAMPLE	*0 CFU/mL*	*450 CFU/mL*	*950 CFU/mL*	*1300 CFU/mL*
No. of Replicates	282	288	288	288
% Correct	100%	100%	100%	100%
Mean A_{450}	0.06	3.10	3.47	3.63
Between Day SD	0.003	0.086	0.083	0.125
Between Day CV	4.83%	2.77%	2.40%	3.44%
Between Run SD	0.002	0.353	0.236	0.176
Between Run CV	3.83%	11.37%	6.80%	4.85%
Within Run SD	0.012	0.535	0.298	0.241
Within Run CV	19.97%	17.23%	8.60%	6.64%
Total SD	0.0126	0.646	0.390	0.324
Total CV	20.9%	20.8%	11.22%	8.9%

D. Reproducibility

The reproducibility of the AMPLICOR MTB Test was determined in a study at three clinical laboratories that had no prior experience in the use of the AMPLICOR Test procedure. A panel consisting of simulated clinical specimens was tested at each laboratory. The simulated specimens were prepared by spiking decontaminated sputum with an avirulent strain of *M. tuberculosis* (ATCC# 25177) The spiked specimens were quantitated by culture and found to contain approximately 0, 450, 950 and 1300 CFU/mL. The results of the testing performed at the 3 sites are presented in Table 7.

Table 7
AMPLICOR Mycobacterium Tuberculosis Test Reproducibility

SITE	*0 CFU/mL*	*450 CFU/mL*	*950 CFU/mL*	*1300 CFU/mL*
Site 1	100% (36/36)	100% (6/6)	100% (6/6)	100% (6/6)
Site 2	100% (36/36)	100% (6/6)	100% (6/6)	100% (6/6)
Site 3	97.2% (35/36)*	100% (6/6)	100% (6/6)	100% (6/6)
Total	99.3 % (143/144)	100% (24/24)	100% (24/24)	100% (24/24)

* Upon repeat, Site 3 had no false positive test results for the 0 CFU/mL specimen

E. Clinical Data

The AMPLICOR MTB Test was evaluated in a prospective multicenter study performed at 10 clinical sites. The 10 sites were geographically and demographically diverse, consisting of 7 large urban medical centers, 1 small community hospital, 1 public health facility, and 1 specialized respiratory center. AFB smear and mycobacterial culture results were obtained for 5797 specimens collected from 2558 patients. Of these, 4156 specimens were collected from 1833 untreated patients. Untreated patients were defined as patients who were antimycobacterial treatment naive, on antimycobacterial treatment for less than 7 days, or had not received antimycobacterial treatment for the previous 12 months. Only specimens collected from untreated patients were used in the data analyses.

Overall, 189 smear positive specimens were collected from 95 untreated patients. Of these specimens, 134 were positive for *M. tuberculosis* complex by the AMPLICOR Mycobacterium Tuberculosis Test and by mycobacterial culture. Four of the MTB culture positive specimens were also MOTT culture positive. Fifty five (55) of the smear positive specimens were negative for *M. tuberculosis* complex by the AMPLICOR Mycobacterium Tuberculosis Test. Of the 55 smear positive/AMPLICOR negative specimens, 7 were culture positive for *M. tuberculosis* complex and 48 were culture negative for *M. tuberculosis* complex. Thirty-nine (39) of the 48 smear positive/MTB culture negative/AMPLICOR negative specimens were MOTT culture positive and included the

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following isolates: MAI complex, *M. kansasii*, *M. xenopi*, *M. fortuitum*, *M. gordonae*, and unidentified rapid growers. The remaining 9 specimens were from patients with all negative mycobacterial cultures.

Table 8 shows the clinical performance of the AMPLICOR Mycobacterium Tuberculosis Test for smear positive specimens obtained from untreated patients as compared to composite culture results for these patients. Table 9 contains the overall clinical performance calculations for the AMPLICOR Mycobacterium Tuberculosis Test and shows the range of results for all 10 clinical sites with 95% Confidence Intervals.

Table 8
AMPLICOR MTB Test Clinical Performance
Smear Positive Specimens Collected from Untreated Patients
Compared to Composite Culture Results

		MTB Composite Culture Result +	MTB Composite Culture Result -
AMPLICOR Result	+	134	0
	-	7	48

Table 9
AMPLICOR MTB Clinical Performance Calculations
Smear Positive Specimens Collected from Untreated Patients
Compared to Composite Culture Results

Parameter	Total Results	Range (10 Sites)	Performance	95% CI
Sensitivity	134/141	88.9 - 100%	95.0%	91.4 - 98.6
Specificity	48/48	100%	100%	97.8 - 100
PPV	134/134	100%	100%	94.0 - 100
NPV	48/55	77.8 - 100%	87.3%	78.5 - 96.1

AMPLICOR MTB TEST TROUBLESHOOTING GUIDE

OBSERVATION	*POSSIBLE CAUSES*	*RECOMMENDED ACTIONS*
Negative Control Values 0.25 A_{450}	Air bubble in well of MWP (well will appear clear in color).	Disperse air bubbles and read MWP again.
	Omission of MWP washing step	Check that the MWP washer is programmed properly. Repeat the test. Check for other possible causes before repeating the test.
	Contamination of reagents.	Discard suspect reagents and repeat entire test using unopened reagents. Check for other possible causes before repeating the test.
	Contamination of equipment or laboratory.	Decontaminate laboratory surfaces and equipment with 10% bleach. Repeat entire test. Check for other possible causes before repeating the test.
Positive Control Values < 2.0 but ≥ 0.35 A_{450}	MWP read more than 1 hour after adding Stop Reagent	Check for brownish precipitate in wells. Repeat MWP detection.
	Conjugate incubation not performed during MWP detection	Repeat entire test. Check for other possible causes before repeating the test.
	Lysis step carried out at > 100°C	Check temperature of heat block and adjust if necessary. Repeat entire test. Check for other possible causes before repeating the test.
	Omission of MWP washing step.	Check that the MWP washer is programmed properly. Repeat entire test. Check for other possible causes before repeating the test.
	Thermal cycler programmed incorrectly.	Check thermal cycler programming and correct if necessary. Repeat entire test. Check for other possible causes before repeating the test.
Positive Control Values < 0.35 A_{450}	Positive Control not added to MicroAmp Tube.	Check volume in MicroAmp tube. If found to be low, repeat entire test. Check for other possible causes before repeating the test.
	Positive Control not added during Stock Preparation.	Repeat entire test. Check for other possible causes before repeating the test.
	Master Mix deteriorated or expired.	Repeat entire test using new reagents. Check for other possible causes before repeating the test.
	Avidin-HRP Reagent deteriorated or expired.	Repeat entire test using new reagents. Check for other possible causes before repeating the test.
	Lysis Reagent not added.	Repeat entire test. Check for other possible causes before repeating the test.
	Lysis Reagent added instead of Neutralization Reagent.	Repeat entire test. Check for other possible causes before repeating the test.
	Neutralization Reagent not added or wrong volume added	Check volume in control tube. If found to be low, repeat entire test. Check for other possible causes before repeating the test.
	Neutralization Reagent deteriorated or expired.	Repeat entire test using new reagents. Check for other possible causes before repeating the test.
	Lysis step carried out at > 100°C	Check temperature of heat block and adjust if necessary. Repeat entire test. Check for other possible causes before repeating the test.
	Thermal cycler programmed incorrectly	Check thermal cycler programming and correct if necessary. Repeat entire test. Check for other possible causes before repeating the test.

AMPLICOR MTB TEST TROUBLESHOOTING GUIDE (continued)

OBSERVATION	*POSSIBLE CAUSES*	*RECOMMENDED ACTIONS*
Specimen Lysis Control Values $0.35 < A_{450}$	Cell Lysis Control not added to MicroAmp Tube.	Check volume in MicroAmp tube. If found to be low, repeat entire test. Check for other possible causes before repeating the test.
	Cell Lysis Control incubated in Neutralization Reagent instead of Lysis Reagent.	Repeat entire test. Check for other possible causes before repeating the test.
	Lysis Reagent added instead of Neutralization Reagent.	Repeat entire assay. Check for other possible causes before repeating the test.
	Lysis Reagent not added.	Repeat entire assay. Check for other possible causes before repeating the test.
	Lysis step carried out at > 100°C	Check temperature of heat block, and adjust if necessary. Repeat entire test. Check for other possible causes before repeating the test.
	Neutralization Reagent not added.	Check volume in control tube. If found to be low, repeat entire test. Check for other possible causes before repeating the test.
	Decreased volume of Neutralization Reagent added.	Check volume in control tube. If found to be low, repeat entire test. Check for other possible causes before repeating the test.
	Neutralization Reagent deteriorated or expired.	Repeat entire test, using new reagents. Check for other possible causes before repeating the test.

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